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From the Department of Epidemiology Blegdamshospitalet Copenhagen Denmark

On the Treatment of Tetanus

By

Bent Møller[1] and Henning Sund Kristensen

During the past 5—10 years there have been great advances in the treatment of tetanus, primarily because tracheotomy and artificial ventilation are being used in many cases. This enables anticonvulsants to be employed more intensively than before. Numerous reports on severe cases of tetanus treated in this more active period are now on record (1, 2, 4, 6, 8, 9, 10, 12). On the other hand the treatment has given rise to serious complications (5, 7, 13). It has not yet been proved to what extent the modern, active and complicated treatment has improved the results. Moreover, it has not been elucidated which anticonvulsants are most suitable for the treatment. In an attempt to throw some light upon these problems the material of non-neonatal tetanus from Blegdamshospitalet, Copenhagen, from the period 1943—1961 was analysed.

Material

The material comprises all non-neonatal cases of tetanus treated in the Department of Epidemiology, Blegdamshospitalet during the period 1943—1961, a total of 124 patients.

The patients were divided into three groups. Within each group the treatment was fairly uniform but different from that used in the other groups.

Group A. Patients treated in 1943—52, a total of 69.

Group B. Patients treated in 1953—56, a total of 24.

Group C. Patients treated in 1957—61, a total of 31.

The *age distribution* is listed in table I which shows that group A included more children than group C which, on the other hand, included a larger number of patients over 70 years of age.

Sex ratio. Table II shows that, as might be expected, there is a male preponderance in all groups.

Severity. An attempt was made to characterize the severity by the incubation period and the period of onset.

According to table III the *incubation period* is approximately equally distributed in groups A, B and C. In about one-quarter of the cases it is unknown.

The *period of onset* we take to mean the time elapsing from the first sign appears until the diagnosis is established. Others have used the period from the first sign until the first *seizure*, but in practice it is difficult to distinguish between marked rigidity and

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TABLE I Age distribution The figures in brackets indicate patients who died

Group	Age in years < 14	14—39	40—69	> 70	Total
A	16 (6)	30 (9)	22 (4)	1 (1)	69 (20)
B	7 (0)	5 (2)	11 (5)	1 (1)	24 (8)
C	3 (0)	9 (2)	13 (1)	6 (1)	31 (4)

mild tonic convulsions Besides, actual convulsions can often be avoided by successful management The period of onset is shown in table IV

On the whole, the distribution by incubation period and period of onset appears to be almost the same in all three groups

Group A includes 4 cases in which the uterus was the site of infection (all with a fatal outcome), while groups B and C include no cases of this type

The material comprises the great majority of cases of non neonatal tetanus occurring in Copenhagen and, in addition patients transferred from hospitals in other parts of Zealand and adjacent islands During the latter part of the period the transferred patients made up a much higher percentage than in the former part, in group C 71 % as compared with 6 % in group A The transport to Blegdamshospitalet was well tolerated but in some cases without premedication or qualified escort, it was unnecessarily risky

Treatment

The treatment of tetanus comprises tetanus antitoxin surgical revision of wounds and antibiotics Moreover, a suitable nutrition and fluid balance must be secured But the most important aspect of the treatment is that which aims directly at reducing or preventing convulsions Since the convulsions as well as the anticonvulsant therapy are apt to give rise to respiratory complications and since these complications — sometimes combined with shock — are usually the *direct cause* of death, the therapeutic attitude to their management is of the utmost importance to the result In this aspect of the management there was a decisive difference before and after 1953

Group A All group A patients but 4 were treated without tracheotomy or artificial ventilation The main anticonvulsant used was chloral, usually administered by the rectal route in doses of 1 g Avertin anaesthesia was used in quite a large number of cases Both were administered in doses which according to modern views are rather small because respiratory depression was then just as dangerous as the convulsions

During a certain period mephenesin was used, without apparently improving the therapeutic results

Group B Since 1953 tracheotomy has been performed in all cases with signs of obstructed airways or insufficient ventilation The widest possible cuff tube was inserted through the fenestra at the 2nd—3rd tracheal ring Artificial ventilation with a Lundia respirator, was performed in nearly all these cases Simultaneously with the active treatment of respiratory complications a far more intensive anticonvulsant therapy was started, partially with drugs which has previously not been used for a prolonged period Nitrous oxide was added to the inspired air and curare was administered by intravenous infusion

Group C Respiratory complications were treated by the same principles as in group B Nitrous oxide was not used and curarization only during short periods while Nembutal was the most used drug in the treatment of the convulsions

Table II Sex ratio The figures in brackets indicate patients who died

Group	Males	Females
A	46 (7)	23 (13)
B	17 (6)	7 (2)
C	24 (4)	7 (0)

Table III Incubation period The figures in brackets indicate patients who died

Incubation period (days)	Group A	Group B	Group C
< 6	2 (2)	5 (3)	3 (2)
6—8	11 (5)	3 (2)	1 (0)
9—12	11 (2)	7 (2)	9 (1)
> 12	19 (3)	4 (0)	9 (1)
Unkrown	26 (8)	5 (1)	9 (0)
Total	69 (20)	24 (8)	31 (4)

Table IV Period of onset (elapsing from the first sign until the diagnosis is established) The figures in brackets indicate the patients who died

Period of onset (days)	Group A	Group B	Group C
< 1	21 (14)	6 (4)	7 (4)
1—2	7 (4)	8 (3)	5 (0)
2—3	9 (0)	2 (0)	6 (0)
> 3	32 (2)	8 (1)	13 (0)
Total	69 (20)	24 (8)	31 (4)

Table V gives the methods of treatment. The *therapeutic principles* employed in group C, and *which are still in use at Blegdamshospitalet are as follows*

As soon as tetanus has been diagnosed, Nembutal is administered in an initial dose of 300 mg to young adults — less to children

Table V Application of the various drugs and of tracheotomy and artificial ventilation. (Number of cases in which the drug or measure concerned was an essential therapeutic factor)

	Group A	Group B	Group C
Chloral	60	15	4
Avertin	24	2	0
Mephenesin	8	1	0
Nitrous oxide	0	8	0
Curare	0	6	3
Nembutal	0	12	28
Other anticonvulsants	13	0	1
Tracheotomy	4	13	16
Artificial ventilation	0	11	12
Total no of pat.	69	24	31

Table VI Approximate hourly dose of Nembutal (in mg/h to an adult person of 70 kg) in various types of tetanus

Incubation period (days)			
< 6	6—8	9—12	> 12
Period of onset (days)			
< 1	1—2	2—3	> 3
102	72	42	30

and elderly persons. Thereafter, a small and constant dose of Nembutal is administered every hour At the outset the treatment is governed mainly by the available data regarding the period of onset and if known, the incubation period Table VI may afford some guidance in the dosage Nembutal is given in a dosage which empirically is at the lower limit of what is needed and supplementary doses are given through the gastric tube intramuscularly or intravenously in the event of increased rigidity or convulsions The hourly *dose is altered only* once

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Group	Age in years < 14	14—39	40—69	> 70	Total
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The treatment of tetanus comprises tetanus antitoxin, surgical revision of wounds and antibiotics Moreover, a suitable nutrition and fluid balance must be secured But the most important aspect of the treatment is that which aims directly at reducing or preventing convulsions Since the convulsions as well as the anticonvulsant therapy are apt to give rise to respiratory complications and since these complications — sometimes combined with shock — are usually the direct cause of death, the therapeutic attitude to their management is of the utmost importance to the result In this aspect of the management there was a decisive difference before and after 1953

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6—8	11 (5)	3 (2)	1 (0)
9—12	11 (2)	7 (2)	9 (1)
12	19 (3)	4 (0)	9 (1)
Unknown	26 (8)	5 (1)	9 (0)
Total	69 (20)	24 (8)	31 (4)

TABLE IV Period of onset (elapsing from the first sign until the diagnosis is established) The figures in brackets indicate the patients who died

Period of onset (days)	Group A	Group B	Group C
< 1	21 (14)	6 (4)	7 (4)
1—2	7 (4)	8 (3)	5 (0)
2—3	9 (0)	2 (0)	6 (0)
> 3	32 (2	8 (1)	13 (0)
Total	69 (20)	24 (8)	31 (4)

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As soon as tetanus has been diagnosed, Nembutal is administered in an initial dose of 300 mg to young adults — less to children and elderly persons Thereafter, a small and constant dose of Nembutal is administered every hour At the outset, the treatment is governed mainly by the available data regarding the period of onset and if known, the incubation period Table VI may afford some guidance in the dosage Nembutal is given in a dosage which empirically is at the lower limit of what is needed and supplementary doses are given through the gastric tube intramuscularly or intravenously in the event of increased rigidity or convulsions The hourly dose is altered only once

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	Group A	Group B	Group C
Chloral	60	15	4
Avertin	24	2	0
Mephenesin	8	1	0
Nitrous oxide	0	8	0
Curare	0	6	3
Nembutal	0	12	28
Other anticonvulsants	13	0	1
Tracheotomy	4	13	16
Artificial ventilation	0	11	12
Total no of pat.	69	24	31

TABLE VI Approximate hourly dose of Nembutal (in mg/h to an adult person of 70 kg) in various types of tetanus

Incubation period (days)			
< 6	6—8	9—12	> 12
Period of onset (days)			
< 1	1—2	2—3	> 3
102	72	42	30

TABLE VII Approximate doses of barbiturate given to patients of various ages in relation to adult dose

Age	Dose	Age (years)	Dose
Newborn—3 mo	0.1	10—14	0.9
3—9 mo	0.2	14—50	1.0
9 mo —2 years	0.3	50—60	0.8
2—4 years	0.4	60—70	0.5
4—6 years	0.6	over 70	0.3
6—10 years	0.8	—	—

TABLE VIII Average stay (in days) in the department for the patients who died The figures in brackets indicate the shortest and longest stay

Group	
A	3.9 (1—8)
B	15.5 (3—29)
C	12.3 (6—24)

in the 24 hours in accordance with the total dose of the previous 24 hours It is difficult to balance between a suitable mitigation of the convulsive tendency on the one hand and an unnecessarily deep barbiturate anaesthesia on the other

In exceptional cases a satisfactory relaxation during the peak of the disease can be obtained only by curarization To this end we used d tubocurarine intravenously or in a few cases intramuscularly Incidentally, short lasting curarization is not infrequently needed during lung physiotherapy surgical *procedures* radiography and the like About two hours after the first administration of Nembutal, a revision of the wound or injured tissues is performed in the presence of an *anaesthetist and otologist* and *at the same* time a gastric tube is passed Tetanus antitoxin is administered and penicillin is given to counteract the tetanus infection

The patient is fed through the indwelling gastric tube with 66 % milk in water To the mixture is added 5 % sugar and 0.2 % sodium chloride 125 ml is given per hour (3) Initially and during periods with a tendency to gastric retention smaller quantities are given Sometimes glucose in water is given instead of the mixture, because it seems to be more easily absorbed

Therapeutic results

Table I shows that the mortality in group A was 29 %, while in group C it was 13 %

From table VIII it is apparent that the patients of groups B and C who died *had been treated for a longer time in the* department than those of the earliest group A (1943—1952)

In group A the causes of death were predominantly convulsions and hypoxic sequelae of convulsions, often associated with hyperpyrexia and shock In a few instances the anticonvulsant entailed too deep anaesthesia Complicating diseases *have apparently been responsible* for death in only two cases, in both of which the uterus was the site of infection

In groups B and C the causes of death are more varied Therefore, all fatal cases of these two groups will be briefly described — in chronological order

1 A boy of 15 died after 28 days' treatment, int al with nitrous oxide *The cause of death was agranulo*cytosis and sepsis
2 A man, aged 48 died at the end of 3 days of severe convulsions during which an emergency tracheotomy was attempted but in vain
3 A woman, aged 53 died after 16 days treatment int al with nitrous

oxide The cause of death was sepsis associated with leukopenia and haemorrhagic diathesis

4 A woman, aged 75, died at the end of 17 days with widespread atelectases This patient had been tracheotomized 6 days after admission, but had not received artificial ventilation

5 A man, aged 56 died of pulmonary emboli after being treated for 19 days Thrombophlebitis had occurred after long continued intravenous infusion

6 A man, aged 50 died of severe pulmonary infection hyperpyrexia and shock at the end of 17 days

7 A man, aged 50 died of large pulmonary emboli, pulmonary infection, hyperpyrexia and shock after 18 days' treatment Thrombophlebitis had developed after long continued intravenous infusion

8 A man aged 69 with aortic stenosis and electrocardiographic signs of coronary insufficiency, died in shock

9 A man aged 71 with a history of posterior wall infarct, died of another infarction this time in the septum

10 A man aged 64, died after 6 days treatment of occlusion of the tracheal tube This occlusion must be ascribed to a too curved tube used at the time

11 A man aged 29 with a fracture of the skull and purulent meningitis, died at the end of 19 days in hyperpyrexia with Ps pyocyaneus pneumonia

12 A man aged 33 died at the end of 24 days of the sequelae of cerebral hypoxia Six days after admission, while curarized, the patient had not been connected with the respirator for 10 minutes When this was discovered cardiac arrest had occurred and was successfully treated with thoracotomy and cardiac massage, but cortical function was completely abolished

Comments and conclusion

The differences in mortality during the three periods (A) 1943—1952 (29 %), (B) 1953—1956 (33 %), and (C) 1957—1961 (13 %) are not statistically significant ($p > 5$) It is evident from table VIII that the patients who died during the period 1957—61 (group C) lived longer than those who died during the period 1943—1952 (group A) This indicates that in fact the treatment was better in group C than in group A

The listed fatal cases from groups B and C indicate that a number of these deaths were due directly to factors related to the new, more active therapy

An analysis of the deaths in 1956 gave the surprising result that nitrous oxide, used for a long period of time has a highly toxic effect upon the bone marrow (7) Presumably, this effect was mainly responsible for two deaths in group B The use of nitrous oxide was then discontinued, and since then our patients have shown no signs of damage to the bone marrow

In two cases permanent intravenous infusions appear to have caused death Fatal thromboembolic complications to long-continued intravenous infusion in patients with tetanus have been described

in the literature (8) In consequence, such infusions were avoided in group C After the fatal case 2 the method for treating life-threatening convulsions was altered Such a case (which is extremely rare) is now treated with curare and ventilation with oxygen by mask or — if this is not possible — through an endotracheal tube

While in group B the cause of death could be attributed to the active therapy in 5 cases, this applies only to 2 cases of group C In one of the latter cases, death was due to obstruction of the airway because of too pronounced curving of the tube, but this factor has now been corrected The other patient died because the connection to the respirator had failed

In other words, there is little doubt that modern, active treatment of tetanus is better than that of former times when it was restricted to combatting the convulsions without any treatment of the respiratory problems arising in the most severe cases

However, the modern treatment is so complicated and specialized and involves so many risks that it warrants restriction to a few centres where a routine can be maintained and experience gained

Summary

The 124 cases of non neonatal tetanus treated in Blegdamshospitalet, Copenhagen, during the period 1943—1961, are analysed The more active treatment of respiratory complications, using tracheotomy and artificial ventilation, permits a more energetic anticonvulsant therapy The mortality is now 13 %, while prior to 1952 it was 29 % The modern therapy has given rise to fatal complications which can presumably best be reduced by concentrating this specialized and exacting task in a very few centres

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Medical Department B (Head A Tybjærg Hansen M D) and Radiological Department (Head Gregers Thomsen M D) Rigshospitalet, Copenhagen Denmark

The Higher Incidence of Valvular Calcification in Males than in Females with Mitral Stenosis

By

K Olesen E Sandoe and C Gudbjerg

Calcification of the mitral valves is a frequent end stage of rheumatic heart disease, and approximately one third of all patients submitted to surgery for the relief of mitral stenosis have calcium deposits on the valve cusps (2, 9, 21, 22) The significance of mitral valvular calcification lies in the fact that the immediate and late results of mitral valvotomy in pure or predominant mitral stenosis are poorer in the presence than in the absence of calcified valves (1, 5 9, 10)

During our investigation of patients with mitral stenosis, which includes tomography for mitral valvular calcification as a routine procedure we have been impressed with the higher incidence of valvular calcification in males than in females Although a similar pattern can be traced in earlier reports in which the valvular calcification was diagnosed by palpation at the time of valvotomy (6 9 15 21 22) the sex difference as regards calcified valves has received little comment (5)

The higher incidence of valvular calcification in males than in females with mitral stenosis is of particular interest when it is related to the increasing evidence that the early and late results of mitral valvotomy tend to be poorer in males than in females (3, 5 13 18)

The objects of this paper are to evaluate the order of magnitude of the male prevalence of calcified valves in mitral stenosis, to comment on its causes and to discuss its implications in analysis of the late results of mitral valvular *surgery*

Material

The series comprises 325 patients with a clinical diagnosis of mitral stenosis admitted to the medical departments of Rigshospitalet, Copenhagen during the years 1955—59 The material consists of 67 males and 258 females with an *age range of 16—69* years (fig 1)

As a routine procedure these patients were examined for mitral valvular calcification by tomography

Comparative material was procured from the literature comprising four series of pa

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Comparative material was procured from the literature comprising four series of pa

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TABLE I. Relationship between valvular calcification and sex in mitral stenosis

Material		Males			Females			Significance of difference	
		Total	+ calcification		Total	+ calcification		χ^2	p value
		n	n	%	n	n	%		
Authors		67	38	*57*	258	53	*21*	33.75	< 0.001
Baden	(1)	47	35	75	120	30	25	32.70	< 0.001
Galloway et al.	(6)	46	25	54	118	28	24	12.82	< 0.001
Turner & Fraser	(22)	48	27	56	202	40	20	21.44	< 0.001
Stewart & Glenn	(21)	43	36	84	116	64	55	9.76	< 0.005
Surgical series combined		184	123	*67*	556	162	*29*	81.55	< 0.001

TABLE II. Incidence of males in total populations of mitral stenosis with and without calcified valves

Material	+ Calcification			− Calcification			Significance of difference	
	Total	Males		Total	Males		χ^2	p value
	n	*n*	%	*n*	*n*	%		
Authors	91	38	*41*	234	29	*12*	33.75	< 0.001
Surgical series combined	285	123	*43*	455	61	*13*	81.55	< 0.001

tients submitted to surgery for mitral stenosis (table I). In these subjects the extent of calcification of the mitral valves was ascertained by surgical palpation during the operation. The series of Baden was operated upon in this hospital during the years 1950—55. The materials of Galloway et al. and Turner and Fraser originate from Great Britain, and the series of Stewart and Glenn comes from U.S.A.

A statistical analysis of the incidence of calcified valves in males and in females and of the sex distribution of the total group with or without valvular calcification is performed. The significance of difference is evaluated by the χ^2 test (Snedecor).

Results

The basic data and the results of the statistical analysis are given in tables I and II. All series show the usual preponderance of females, the average ratio males : females being 1 : 3. The trends observed are as follows.

1. Incidence of calcified valves in males and females

In our series tomography revealed mitral valvular calcification in 38 out of 67 males (57 %) and in 53 out of 258 fe-

males (21 %) A similar degree of prevalence in males is present in the four series with operatively verified calcification (table I) In the combined surgical series calcified valves occurred in 67 % of the males and in 29 % of the females

In all groups the incidence of calcified valves is significantly higher in males than in females ($p < 0.001$)

2 Sex distribution in groups with or without calcification

In our series the males make up 41 % of the total group with valvular calcification and 12 % only of the group without calcified valves Similarly, in the combined surgical series the males amount to 43 % of the group with calcification and to 13 % only of the group without calcified valves (table II)

In both series the incidence of males is significantly higher in the group with calcified valves than in the group without ($p < 0.001$)

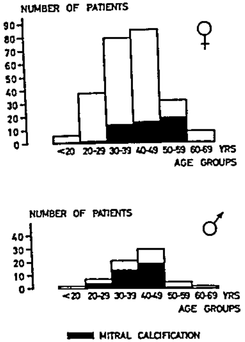


Fig 1 Age and sex distribution of authors series of mitral stenosis Note the higher incidence and the earlier appearance of calcified valves in the males

3 Age and valvular calcification (fig 1)

In our total series the mean age of males was 40 8 years and that of females 41 9 years For patients with valvular calcification the mean age of males amounted to 39 4 years and that of females to 47 3 years

Apparently the prevalence of calcified valves in males cannot be explained by age differences between the sex groups The findings might rather suggest an earlier appearence of calcified valves in males than in females

Discussion

The calcification of the mitral cusps in patients with mitral stenosis is usually explained as a consequence of previous rheumatic infection The calcium is found in the valve cusps and in the fused commissures, and extension into the annulus fibrosus is relatively uncommon (4) This type of calcification should be distinguished from the calcification of the mitral annulus fibrosus which in the absence of previous rheumatic infection may be found in the otherwise normal heart after middle age and which is more common in females (2 4 8, 20) Such calcification is rarely functionally significant although massive calcification of this type causing anatomical deformity of the mitral orifice has been reported (12)

The findings in our tomographic series and the consistent trends in the

four series with operatively verified calcification establish beyond doubt that calcified valves in mitral stenosis are more than twice as frequent in males as in females. The prevalence of valvular calcification in males is not easily explained. It can hardly be related to the severity of the rheumatic infection since the incidence of clinically recognized rheumatic infection or the number of rheumatic recurrences are not significantly higher in males than in females with mitral stenosis (5, 16). Nor does age seem a factor involved since in our series the average age of males with calcified valves was lower than that of females. A tentative explanation might be that males with mitral stenosis manifest a more marked tendency to valvular calcification parallel to the earlier and more extensive arteriosclerotic calcification in the male than in the female sex which has been found in diabetics (11) and in hospitalized patients in general (14, 17).

The calcified valves in mitral stenosis present a challenge to the surgeon. Although even marked calcification does not preclude a good operative result it is generally agreed that the incidence of incomplete valvotomies is higher in the presence than in the absence of calcified valves (1, 9, 21). It is in full accordance with this experience that longterm follow-up indicate that the postoperative improvement and the survival rates after valvotomy are poorer in patients with calcified valves than in those without (1, 5, 9, 10).

From the data presented above it appears that males make up more than 40 % of the patients with calcified valves but only 12 % of the population without calcification. The significantly higher incidence of males in the population with calcified valves than in the group without calcification is of particular interest when it is related to the accumulating evidence that males apparently derive less benefit from mitral valvotomy than females (3, 5, 13, 18). These facts may suggest that prevalence of valvular calcification in males is a major cause of the poorer postoperative results in the male sex. Since the follow-ups available at present are inadequate to answer this problem it is our purpose to call attention to the higher incidence of valvular calcification in males than in females with mitral stenosis, and to suggest that this sex difference is taken into consideration in future analysis of the late results of mitral valvotomy.

Summary

Tomography for mitral valvular calcification in patients with mitral stenosis revealed calcified valves in 38 out of 67 males (57 %) and in 53 out of 258 females (21 %). Accordingly, the males represented 41 % of the cases with calcified valves but 12 % only of the cases without calcification. Exactly the same trends for a male prevalence of calcified valves could be shown in earlier reports with operatively verified valvular calcification.

The significantly higher incidence of valvular calcification in males than in females with mitral stenosis cannot be related to sex differences in the severity of rheumatic infection or in age, but is tentatively ascribed to a more pronounced tendency to calcification in the male sex.

The prevalence of calcified valves in males with mitral stenosis is of prognostic significance, since an increasing number of reports indicate that the immediate and late results of mitral valvotomy are poorer in the presence than in the absence of calcified valves. At the same time evidence is accumulating that the benefit from mitral valvotomy is less in males than in females. We call attention to the higher incidence of calcified valves in males than in females with mitral stenosis as a possible explanation of the poorer postoperative results in males, and suggest that this sex difference is taken into consideration in future analysis of the results of mitral valvotomy.

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Department VIII Ullevål Hospital (Head A Jervell M D) and Life Insurance Companies Institute for Medical Statistics at the Oslo City Hospitals (Report no 7) (Head Knut Westlund M D), Oslo, Norway

Coronary Heart Disease and Serum Cholesterol in Males in Different Parts of Norway

By

A JERVELL, KIRSTEN MEYER (†) and K WESTLUND

It is generally accepted that the incidence of coronary heart disease in Norway, as in many other countries, has increased since the second world war, particularly in males The mortality statistics indicate however, that this increase has not been equally large in all parts of the country (4, 7) It appears that type of habitation is more important than geographic region Towns suburbs, and other densely populated areas generally have a higher mortality than is found in areas with a scattered population Table I gives an example of the relationships which have been found Towns are contrasted with sparsely populated rural municipalities for males in 3 age ranges

Sparsely populated municipalities show a considerably lower mortality than towns This is true for the total mortality, but is more pronounced for cardio-vascular diseases The table also shows that the difference for malignant tumours is proportionally about the same as for total mortality

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It is difficult to assess the extent to which such a comparison reflects true morbidity relationships It is reasonable to assume that the figures for total mortality are very nearly correct The Central Bureau of Statistics refers all deaths to the municipality of residence It is admittedly easy to find examples of persons who moved shortly prior to death, and possibly because of the illness but it is hard to believe that this could cause a bias large enough to affect the interpretation of the differences in table I It is more hazardous to evaluate the relationships of the individual causes of death Medical supervision of the population is on the whole less intense in sparsely populated areas than in towns, and it is to be expected that the diagnosis will often be less specific, both in morbidity and in mortality statements But it seems unwise to reject differences such as those in table I on the basis of this general argument It has repeatedly been shown that geographic or secular differ

TABLE I Mortality in towns and in sparsely populated municipalities in Norway, in 1954—57 (males)

Cause group[1]	Age 40—49	50—59	60—69
	Deaths per 10 000 per year		
All causes			
Towns	38 8	110 4	269 5
Sparsely populated municip	*29 5*	*69 6*	*176 8*
Cardio-vascular diseases (330—334 400—468, 592)			
Towns	12 2	45 9	139 2
Sparsely populated municip	7 0	26 2	56 2
Malignant tumours (140—205)			
Towns	8 0	28 6	65 4
Sparsely populated municip	6 5	17 3	48 0
	Mortality as % of mortality in towns		
All causes			
Towns	100	100	100
Sparsely populated municip	76	63	66
Cardio vascular diseases			
Towns	100	100	100
Sparsely populated municip	57	57	60
Malignant tumours			
Towns	100	100	100
Sparsely poulated municip	*81*	*60*	*73*

[1] Numbers refer to the International Statistical Classification

ences in mortality from lung cancer, multiple sclerosis, and coronary heart disease have reflected a real and epidemiologically *interesting difference* in incidence

When pursuing a contrast like that of table I one would first of all look around for areas which might give an even sharper contrast One difficulty is that numbers soon may become too small In what follows we will describe a comparison of the mortality in Oslo with 7 rural municipalities in northern Gudbrandsdal (Dovre, Heidal, Lesja, Lom, Sel, Skjåk, and Vågå) and with 12 rural municipalities on the coast of Möre og Romsdal (Brattvær, Bud, Edöy, Frena, *Giske, Heroy, Hopen, Hustad, Nord* Aukra, Sandöy, Sor Aukra, and Vigra)

The 7 municipalities in Gudbrandsdal are *thought to be characteristically Norwegian* mountain valleys Tradition is important A diversified agriculture is the main source of income The produce is mostly potatoes and

barley Other grains do not ripen in many places All farms have cows many have sheep, while goats are on the decline Pigs are kept, particularly in Skjåk. Cheese is manufactured and there are some sawmills and carpentry shops Large forests provide work for many

The age specific mortality in Gudbrandsdal was first analyzed almost a century ago for the years 1856—65 and found to be very much lower than the Oslo mortality

The 12 coastal municipalities in Möre og Romsdal were selected with a view to getting an area with a relatively large fishing population No prior information as to the mortality level influenced the choice of this area

Table II shows the distribution of the economically active male population in 1950 and in 1960 according to industry in the three areas Fig 1 illustrates the location of the study areas

The mortality comparison will be limited to males aged 40—69 As the population is small in both rural areas we have studied a ten year period 1952—61 Table III shows mortality per 10 000 per year with absolute numbers of deaths in parentheses In table IV the mortality in the two rural areas is given in per cent of the Oslo mortality

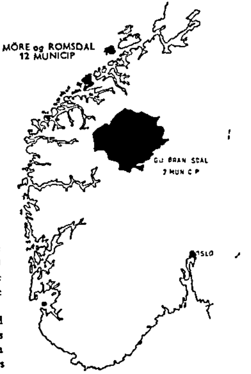


Fig 1 Areas included in mortality comparison

The relationship between Oslo and the 12 coastal municipalities in Möre og Romsdal is approximately the same as the relationship in table I between Oslo and all sparsely populated municipalities in Norway This holds not only for the total mortality but for cardio-vascular diseases and malignant tumours as well

In the 7 Gudbrandsdal municipalities however mortality is extraordinarily low Total mortality is about half that of Oslo It is of interest to note that in 1856—1865 the mortality in Gudbrandsdal in the three age groups studied was very nearly the same as the present mortality in Oslo

For cardio-vascular diseases and for malignant tumours the contrast between Gudbrandsdal and Oslo is still more marked than for all causes. For cause group 420—422 the Gudbrandsdal rate is about one fifth of the Oslo rate at age 40—59 Strictly speaking group number 421 does not belong in this context Its inclusion makes no practical difference however A study of the death certificates — for what it is worth — gave no support to the idea that the low mortality from arteriosclerotic and degenerative heart disease in Gudbrandsdal is due to a diagnostic transfer to other causes of death Conversely there is no reason to believe that the difference in heart disease mortality between Oslo and Gudbrandsdal can be ascribed to excess certification in Oslo The Life Insurance Companies Institute for Medical Statistics at the Oslo City Hospitals (8) investigated male deaths at age 30—64 in Oslo in 1956—57 Of 417 deaths coded by the Central Bureau of Statistics to 420 1 there were only 12 cases where the diagnosis of myocardial infarction old or new could be considered unlikely in view of the information available Nevertheless the true relationship between

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All causes			
Towns	38.9	110.4	269.5
Sparsely populated municip.	29.5	69.6	178.9
Cardio-vascular diseases (330—334, 400—468, 592)			
Towns	12.2	45.9	159.2
Sparsely populated municip.	7.0	26.2	95.2
Malignant tumours (140—205)			
Towns	8.0	25.6	65.4
Sparsely populated municip.	6.5	17.5	49.0
	Mortality as % of mortality in towns		
All causes			
Towns	100	100	100
Sparsely populated municip.	76	63	66
Cardio-vascular diseases			
Towns	100	100	100
Sparsely populated municip.	57	57	60
Malignant tumours			
Towns	100	100	100
Sparsely populated municip.	81	68	75

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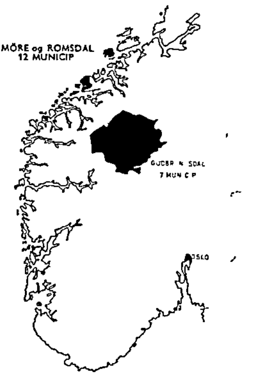


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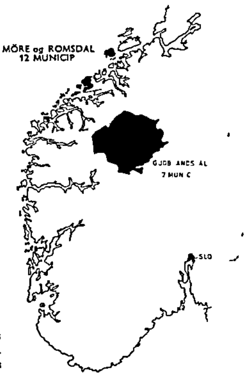


Fig 1 Areas included in mortality comparison

TABLE II. Economically active male population. Percentage distribution by industry, in 1950 and 1960

	Oslo		12 rural municip. in Møre og Romsdal		7 rural municip. in Gudbrandsdal	
	1950	1960	1950	1960	1950	1960
Agriculture and forestry	1	1	24	19	54	51
Fishing and whaling	0	0	41	42	0	0
Manufacturing	34	32	11	12	12	10
Other	65	67	24	27	34	39
	100	100	100	100	100	100

TABLE III. Mortality in Oslo, in 12 rural municipalities in Møre og Romsdal and in 7 rural municipalities in Gudbrandsdal during 1952—61 (males). Deaths per 10,000 per year. In parentheses: Number of deaths during 1952—61

Cause group[1]	Age 40—49		50—59		60—69	
All causes						
Oslo	40.0	(1,313)	114.0	(3,433)	302.9	(5,817)
Møre og Romsdal (12 municip.)	29.8	(55)	73.7	(114)	175.6	(174)
Gudbrandsdal (7 municip.)	26.8	(36)	45.0	(59)	139.3	(138)
Cardio-vascular diseases (330—334, 400—468, 592)						
Oslo	13.5	(442)	49.8	(1,501)	151.2	(2,904)
Møre og Romsdal	8.1	(15)	30.4	(47)	90.8	(90)
Gudbrandsdal	5.2	(7)	15.2	(20)	62.6	(62)
Arteriosclerotic and degenerative heart disease (420—422)						
Oslo	8.3	(274)	33.5	(1,010)	93.6	(1,799)
Møre og Romsdal	4.3	(8)	18.1	(28)	57.5	(57)
Gudbrandsdal	1.5	(2)	6.1	(8)	32.3	(32)
Arteriosclerotic and degenerative heart disease + unknown cause (420—422, 795)						
Oslo	10.6	(349)	38.8	(1,163)	104.9	(2,014)
Møre og Romsdal	6.0	(11)	20.7	(32)	64.6	(64)
Gudbrandsdal	3.7	(5)	10.7	(14)	37.3	(37)
Malignant tumours (140—205)						
Oslo	8.9	(291)	29.0	(873)	78.6	(1,509)
Møre og Romsdal	6.5	(12)	20.0	(31)	46.4	(46)
Gudbrandsdal	3.7	(5)	7.6	(10)	38.3	(38)

[1] Numbers refer to International Statistical Classification.

TABLE IV Mortality in 12 rural municipalities in More og Romsdal and in 7 rural municipalities in Gudbrandsdal during 1952—61 (males) as per cent of mortality in Oslo

Cause group[1]	Age 40—49	50—59	60—69
All causes			
Oslo	100	100	100
More og Romsdal	74	65	58
Gudbrandsdal	67	39	46
Cardio-vascular diseases (330—334 400—468 592)			
Oslo	100	100	100
More og Romsdal	60	61	60
Gudbrandsdal	39	31	41
Arteriosclerotic and degenerative heart disease (420—422)			
Oslo	100	100	100
More og Romsdal	52	54	61
Gudbrandsdal	18	18	35
Arteriosclerotic and degenerative heart disease + unknown cause (420—422 795)			
Oslo	100	100	100
Möre og Romsdal	57	53	62
Gudbrandsdal	35	28	36
Malignant tumours (140—205)			
Oslo	100	100	100
More og Romsdal	73	69	59
Gudbrandsdal	42	26	49

[1] Numbers refer to International Statistical Classification

Oslo and Gudbrandsdal with respect to coronary heart disease is perhaps best estimated by adding group 795 — ill-defined and unknown causes — to the group 420—422

Table IV shows that for the group 420—422 + 795 the mortality in Gudbrandsdal is roughly one third of the mortality in Oslo in all three age groups Table III shows that the mortality in Gudbrandsdal is very nearly the same as the mortality in Oslo for males 10 years younger

Among the 95 deaths at age 40—59 in Gudbrandsdal there were 25 deaths from accidents and suicide The expected number of such deaths was 21 8 on the basis of the age specific rates in Oslo.

In the same age interval there were in Gudbrandsdal 3 deaths ascribed to multiple sclerosis against 0 9 expected on an Oslo basis A fourth certificate gave multiple sclerosis as a contributory cause

The serum cholesterol level in a community has been found to be closely associated with mortality from coronary heart disease It is therefore of interest to compare our three geographical areas with respect to serum cholesterol The comparison will be limited to males age 40—59

From Oslo a large material is available Berge and Nicolaysen (1) have reported the results of a study of nearly 7 000 males who were under supervision by industrial physici

TABLE II Economically active male population Percentage distribution by industry, in 1950 and 1960

	Oslo		12 rural municip in More og Romsdal		7 rural municip in Gudbrandsdal	
	1950	1960	1950	1960	1950	1960
Agriculture and forestry	1	1	24	19	54	51
Fishing and whaling	0	0	41	42	0	0
Manufacturing	34	32	11	12	12	10
Other	65	67	24	27	34	39
	100	100	100	100	100	100

TABLE III Mortality in Oslo, in 12 rural municipalities in More og Romsdal, and in 7 rural municipalities in Gudbrandsdal during 1952—61 (males) Deaths per 10 000 per year In parentheses Number of deaths during 1952—61

Cause group[1]	Age 40—49		50—59		60—69	
All causes						
Oslo	40 0	(1,313)	114 0	(3,433)	302 9	(5,817)
More og Romsdal (12 municip)	29 8	(55)	73 7	(114)	175 6	(174)
Gudbrandsdal (7 municip)	26 8	(36)	45 0	(59)	139 3	(138)
Cardio vascular diseases (330—334, 400—468, 592)						
Oslo	13 5	(442)	49 8	(1,501)	151 2	(2,904)
More og Romsdal	8 1	(15)	30 4	(47)	90 8	(90)
Gudbrandsdal	5 2	(7)	15 2	(20)	62 6	(62)
Arteriosclerotic and degenerative heart disease (420—422)						
Oslo	8 3	(274)	33 5	(1 010)	93 6	(1 798)
More og Romsdal	4 3	(8)	18 1	(28)	57 5	(57)
Gudbrandsdal	1 5	(2)	6 1	(8)	32 3	(32)
Arteriosclerotic and degenerative heart disease + unknown cause (420—422, 795)						
Oslo	10 6	(349)	38 8	(1 168)	104 9	(2 014)
More og Romsdal	6 0	(11)	20 7	(32)	64 6	(64)
Gudbrandsdal	3 7	(5)	10 7	(14)	37 3	(37)
Malignant tumours (140—205)						
Oslo	8 9	(291)	29 0	(873)	78 6	(1 509)
More og Romsdal	6 5	(12)	20 0	(31)	46 4	(46)
Gudbrandsdal	3 7	(5)	7 6	(10)	38 3	(38)

[1] Numbers refer to International Statistical Classification

infarction (3) The blood was drawn more than half a year after the infarction in all cases

All blood samples from all four groups were examined in the Johan Throne Holst s Institute for Nutrition Research Oslo by the method of Hauge and Nicolaysen (2) The results are shown in table V

It appears that the mean serum cholesterol was as follows in these four groups of males age 40—59

Infarction patients 299 mg %, Oslo 270 mg %, Möre og Romsdal 258 mg %, and Gudbrandsdal 223 mg %

Fig 2 illustrates the striking difference in the proportion of men having below 225 mg % or above 275 mg %

In order to see if the low values in Gudbrandsdal were determined by the season of the blood test we re-examined 98 men in Lom Skjåk after 6 months i e in December 1960 The mean in June 1960 for these men was 222 5 mg % In December the mean was 219 9 mg % Seasonal variation is therefore hardly of importance when evaluating the differences in table V

The serum cholesterol level in Möre og Romsdal lies between those of Oslo and Gudbrandsdal A comparison of tables IV and V shows however that there is no linear relationship between mean serum cholesterol and mortality from arteriosclerotic and degenerative heart disease in the three areas Rather the proportion of men with cholesterol 300 mg % or above varies roughly with the mortality rate Naturally one would not expect serum cholesterol to be the sole determinant of mortality In addition interpretation is complicated by errors in mortality rates and the non random sampling of subjects for blood testing

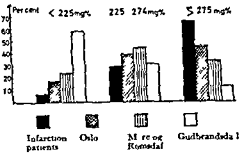


Fig 2 Percentage distribution of serum cholesterol in four groups of men age 40—59 years

If one postulates that the cholesterol distribution for infarction patients in table V is representative of the situation prior to the outbreak of arteriosclerotic heart disease and if the industrial physicians material is representative of males without coronary heart disease in Oslo, it is possible to estimate the relative risk of getting arteriosclerotic heart disease at different cholesterol levels If the risk for serum cholesterol below 225 mg % is put equal to 1, the risk will be 1 9 at 225—274 mg %, and 3 8 at 275 mg % or above These relative risks, in conjunction with the cholesterol distributions in Gudbrandsdal and Oslo, lead to an expected incidence in Gudbrandsdal which is about 60 % of the Oslo incidence We have just concluded, however, that the mortality from arteriosclerotic heart disease in Gudbrandsdal is only 1/3 of the Oslo mortality Some of the possible reasons for such a discrepancy have already been mentioned Another possibility is that the serum cholesterol for infarction patients in table V may tend to be lower than before the onset of disease This would mean that the relative risk estimates are too low for 225—274 mg % and 275 mg % or above A prospective study, at present under way in Oslo, may clarify this point Further it is possible that a few years ago the cholesterol distribution difference between Oslo and Gudbrandsdal was even more pronounc

TABLE V. Distribution of serum cholesterol in 4 groups (males, age 40—59)

Serum cholesterol (mg %)	Number of men: Oslo, Infarction patients	Oslo, Industrial physicians material	Möre og Romsdal, Fishermen	Gudbrandsdal	Percentage distribution: Oslo, Infarction patients		Oslo, Industrial physicians' material		Möre og Romsdal, Fishermen		Gudbrandsdal	
—124	0	8	0	2	0		.1		0		.6	
125—149	0	20	2	10	0		.3		1.4		3.1	
150—174	2	91	5	28	.7	6.6	1.4	17.1	3.4	24.1	8.7	57.5
175—199	6	298	9	59	2.2		4.7		6.2		18.3	
200—224	10	675	19	86	3.6		10.6		13.1		26.7	
225—249	36	1,111	28	60	13.1	28.8	17.5	38.6	19.3	43.5	18.6	30.4
250—274	43	1,337	35	33	15.7		21.1		24.2		11.8	
275—299	51	1,177	26	20	18.6		18.5		17.9		6.2	
300—324	48	819	9	11	17.6		12.9		6.2		3.4	
325—349	30	440	4	5	10.9	64.6	6.9	44.3	2.8	32.4	1.6	12.1
350—374	23	223	5	3	8.4		3.5		3.4		1.0	
375+	25	158	3	0	9.2		2.5		2.1		0	
	274	6,357	145	322	100.0		100.0		100.0		100.0	
Arithmetic mean	298.7	270.2	257.6	222.8								

ans. Here we will reproduce the distribution found for the 6,357 men who at the time of the blood test had no diagnosed diabetes, coronary heart disease, intermittent claudication or apoplexy, and who had a diastolic blood pressure not exceeding 100 mm Hg.

In 4 of the municipalities in Gudbrandsdal (Lom, Skjåk, Lesja, and Dovre) we have obtained serum cholesterol determinations for 322 males. This is slightly more than 20 % of all males in the age group 40—59 in the 4 municipalities. The blood was drawn in June 1960 in Lom Skjåk and in April—May 1960 in Lesja Dovre. No formal sampling procedure was used. By travelling through the area contact was established with men encountered on the roads, in the fields, in sawmills, in shops, etc. None of the men was known to us beforehand. None of them had a diagnosed diabetes or obvious symptoms of hyper- or hypothyroidism.

In Möre og Romsdal the blood samples were obtained in February—May 1963 in connection with the obligatory medical examination of fishermen prior to departure for the seasonal fisheries. It should be noted that the men do not necessarily belong to one of the 12 municipalities included in the mortality analysis.

For comparison we also give the serum cholesterol distribution for 274 males in Oslo, age 40—59, who had suffered a myocardial

TABLE VII Characteristics of 22 males in Lom and Skjåk whose diet was examined in April 1961

	Full time farmers or forest workers	Others	Total
No	10	12	22
Mean			
Age	53	52	53
Height (cm)	174	172	173
Weight (kg)	76	78	77
Non-smokers	2	3	5
Cigarette-smokers	1	5	6
Users of other forms of tobacco only	7	4	11
Mean serum cholesterol April 1961	202 0	240 1	222 8
Mean intake			
Fats (g)	124	138	132
Carbohydrates (g)	377	290	330
Proteins (g)	109	91	99
Total calories	3 065	2 769	2 904
Percentage calories from fat	36 6	45 2	41 3
Percentage polyunsaturated fat	8 3	7 3	7 8

TABLE VIII Product moment correlations 22 males in Lom and Skjåk

	r_{xy}	r_{xz}	r_{xy}	$r_{xz.y}$	r_{xu}	r_{xv}	$r_{xu.v}$	$r_{xv.u}$
All 22 men	− 14	[1] − 48	+ 10	[1] − 47	+ 39	− 24	+ 32	− 02
10 full time farmers or forest workers	− 29	+ 11	− 37	+ 27	− 38	− 03	− 47	− 30

[1] Significant at the 5 % level
x Mean serum cholesterol April 1961
y Fat intake in g
z Carbohydrate intake in g
u Percentage calories from fat
v Percentage polyunsaturated fat

were made in April 1961 for the selected men In 19 cases two samples were obtained about 10 days apart In the remaining 3 cases only one sample was examined in April From some of the men an additional sample was taken in June 1961 In this paper only the mean April values will be considered For most of the men this mean differed only slightly from the mean of all samples from the period June 1960 — June 1961

On the basis of their occupation the men have been divided in the same way as the Gudbrandsdal men in table VI

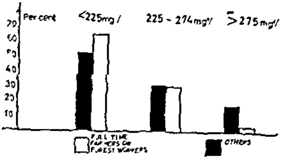


Fig 3 Percentage distribution of serum cholesterol in two groups of men, age 40—59, in Gudbrandsdal.

ed than in 1960, and that the mortality observed in this study is mainly a function of this earlier cholesterol differential

The Gudbrandsdal sample was subdivided on basis of occupation 128 men who were full time farmers or forest workers had a mean serum cholesterol of 211 5 mg % The remaining 194 men had a mean of 230 2 mg % Table VI and fig 3 show the distribution in the two groups

It appears that the farming population, which has preserved more fully the traditional way of living, has a very small proportion at 275 mg % or above The contrast presented by the other group indicates perhaps that the serum cholesterol distribution is in the process of shifting towards higher levels for Gudbrandsdal males as a whole

The composition of the diet in Gudbrandsdal is obviously of interest In April 1961 the National Society for Nutrition and Health made a study of the diet of 22 men in the age range 40—59 from two of the rural municipalities (Lom and Skjåk) discussed here The study has been reported in full by Nes et al (6) Here, some of the findings will be related to the serum cholesterol of the men

The subjects in the dietary study were selected from among the 98 men whose serum cholesterol had been examined both in June and December 1960 Some men with low values in both tests, some with high and some with widely varying values were selected Of 30 men originally selected 8 were omitted for various reasons The serum cholesterol levels were not known to the diet investigators

In connection with the dietary study additional serum cholesterol analyses

TABLE VI Distribution of serum cholesterol in 2 occupational groups Gudbrandsdal, males age 40—59

Serum cholesterol (mg %)	Number of men: Full time farmers or forest workers	Number of men: Others	Percentage distribution: Full time farmers or forest workers	Percentage distribution: Others
< 225	84	101	65 6	52 1
225—274	39	59	30 5	30 4
> 275	5	34	3 9	17 5
	128	194	100 0	100 0
Arithmetic mean	211 5	230 2		

TABLE VII Characteristics of 22 males in Lom and Skjåk whose diet was examined in April 1961

	Full time farmers or forest workers	Others	Total
No	10	12	22
Mean			
Age	53	52	53
Height (cm)	174	172	173
Weight (kg)	76	78	77
Non-smokers	2	3	5
Cigarette-smokers	1	5	6
Users of other forms of tobacco only	7	4	11
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All 22 men	− 14	[1]− 48	+ 10	[1]− 47	+ 39	− 24	+ 32	− 02
10 full time farmers or forest workers	− 29	+ 11	− 37	+ 27	− 38	− 03	− 47	− 30

[1] Significant at the 5 % level
x Mean serum cholesterol April 1961
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On the basis of their occupation the men have been divided in the same way as the Gudbrandsdal men in table VI

10 men were working full-time in farming and forest work, while 12 held other jobs In the latter group, however, 5 did part-time farming

Some characteristics of the two groups are set out in table VII It is seen that the two groups are similar in age, height and weight

Mean cholesterol shows a large difference (although barely significant at the 5 % level) between the two occupational groups For the 22 men as a whole the mean is the same as for the 322 men from Gudbrandsdal in table V

Even without a control group it is apparent that the tobacco consumption in Gudbrandsdal is different from that of city dwellers

Dietary components differ in the two subgroups The group with low mean cholesterol has lower mean fat intake, higher intake of carbohydrates and proteins, higher percentage of polyunsaturated fat, and higher total calorie intake than in the other group The largest difference is to be found in the consumption of carbohydrates

The relationship in individuals between serum cholesterol and characteristics of the diet has been examined by means of correlation coefficients, total and partial, as shown in table VIII

There is no correlation between fat intake in grams and serum cholesterol In the total group there is a significant negative correlation between cholesterol and carbohydrate intake, which also holds when grams of fat is kept constant ($r_{xz\cdot y}$) Correspondingly, there is a positive correlation between serum cholesterol and percentage calories from fat

However, there is no indication of any association between cholesterol and food components within the group of full time farmers or forest workers That is, the associations in the total group may be explained by the difference in mean values between the two occupational subgroups

The correlation coefficients remained essentially the same when one man who had previously suffered an infarction was omitted from the analysis

Analysis in terms of Kendall's rank correlation coefficient led to the same conclusions

The absence of associations within a group of men which is homogenous with respect to both locality and occupation is in conformity with the findings in bank men by Morris et al (5) The present data are of interest, however, in that they show quite pronounced differences in diet and serum cholesterol between two groups of men which constitutionally must be very similar and who live in the same rural area

Summary

During 1952—61 the mortality from all causes in middle-aged males in 7 rural municipalities in Gudbrandsdal was about half that in Oslo For arteriosclerotic and degenerative heart disease, and for malignant tumors, the difference was still more pronounced with rates in Gudbrandsdal about 1/3 of Oslo rates

Twelve coastal municipalities in More og Romsdal occupied an intermediate position

The differences in mortality from arteriosclerotic and degenerative heart disease were related to differences in serum cholesterol distributions for males aged 40—59 from the same areas For comparison the serum cholesterol distribution is also given for patients from Oslo with myocardial infarction and of the same age All cholesterol analyses have been performed at the Institute for Nutrition Research in Oslo Table V and fig 2 show that the distributions are very different for the 4 groups of men It seems difficult however, to "explain the entire mortality difference between Oslo and Gudbrandsdal purely in terms of serum cholesterol levels

The National Society for Nutrition and Health has studied the diet of 22 men in Gudbrandsdal The serum cholesterol of these men has been correlated with consumption of main dietary components There was a significant negative association between serum cholesterol and carbohydrate consumption However, when the 22 men were divided in two groups according to occupation, it was found that men who worked exclusively as farmers had a lower mean serum cholesterol, higher carbohydrate and lower fat intake than the others Within occupational groups no association was found between serum cholesterol and dietary components

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Effect of Synthetic ACTH on Steroid Hormone Production in Man

By

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The ACTH molecule has been found to contain 39 amino acids in sheep, pig and man (2, 9) Moreover, in ACTH from these three species the first 24 amino acids in the chain and the last 6 ones are identical, whereas minor differences exist with respect to amino acids 25—33 (3) Thus, from a chemical point of view it seemed possible that the initial part of the ACTH molecule plays a key role for the biological activities of ACTH and there are in fact experimental results to support such a hypothesis (10) Recently Kappeler and Schwyzer (6) synthesized a peptide consisting of 24 amino acids and identical with the first part of natural ACTH It then became possible to evaluate the steroidogenic effects of this synthetic beta[1 24] corticotropic hormone as compared to naturally occurring ACTH Accordingly, we have investigated the effect of the two ACTH preparations on plasma cortisol level and on urinary excretion of 17 ketogenic steroids of certain tetrahydro derivatives and of total and partially fractionated 17 ketosteroids, in addition the influence of synthetic ACTH on urinary aldosterone excretion was studied in two cases

Submitted for publication May 2? 1964

Material and methods

The studies were performed in 9 hospitalized patients as presented in table I No medication other than ACTH was given Both ACTH preparations were given to each patient at least 6 days being allowed between the two infusions to ensure that the adrenal had returned to normal status Both preparations—natural highly purified ACTH prepared from pig pituitaries (cortrophin Pharmacia) and synthetic ACTH (beta[1 24] corticotropin CIBA) — were given through infusion of isotonic glucose or saline over 8 hours The doses used were 70 IU (new units) for natural ACTH and 0 25 mg for the synthetic compound the biological activity of the synthetic ACTH has been reported to be 106 USP—U/mg (13) For the determination of plasma cortisol blood was drawn from the anticubital vein before and at various intervals during (and occasionally after) the ACTH infusion Urinary steroid excretion as a rule was estimated by

TABLE II Effects of natural (Nat.) and synthetic (Synth.) ACTH on urinary 17 ketogenic steroids During the day of ACTH infusion urine was collected for periods varying between 4 and 11 hours Figures within ( ) represent excretion calculated for one hour

		MG 17 ketogenic steroids						
		Day before	Day of *infusion (hours)*					Day after
Pat	ACTH	(total)	7—12	12—16	16—20	20—7	Total	(total)
BAS	Nat	19.3 (0.81)	4.1 (0.82)	9.0 (2.25)	9.4 (2.36)	18.9 (1.72)	41.7 (1.74)	18.8 (0.78)
	Synth	*15.0* (0.63)	*4.9* (0.98)	*6.0* (1.50)	*10.4* (2.60)	*19.6* (1.73)	*41.6* (1.73)	15.5 (0.65)
AGW	Nat	16.5 (0.69)	8.8 (1.76)	24.0 (6.0)	25.5 (6.37)	29.2 (2.66)	87.5 (3.73)	25.6 (1.06)
	Synth	17.2 (0.72)	15.2 (3.02)	31.2 (7.80)	19.3 (4.83)	27.4 (2.49)	93.1 (3.88)	28.1 (1.17)

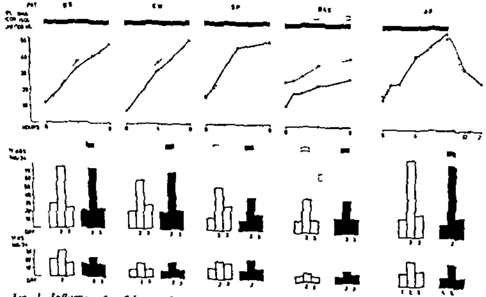


FIG. 1 Influence of an 8-hour infusion of synthetic and natural ACTH on plasma cortisol urinary 17 ketogenic steroids (17 KGS) and 17 ketosteroids (17 KS)

Synthetic ACTH

Natural ACTH

TABLE I Summary of pertinent clinical data

Pat	Age	Sex	Body weight (kg)	Clinical diagnosis and comments
BS	19	♂	93	Obesity + decreased glucose tolerance
FW	56	♀	87	Obesity
SP	41	♀	56	Pituitary tumor with amenorrhea but without thyroid or adrenocortical insufficiency
BAS	26	♀	30	Fanconi syndrome
AGW	32	♀	65	Cushing's syndrome 3 months after implantation of Y^{90} in the sella turcica
IJ	32	♀	57	Hypertrichosis without endocrine disease
ML	40	♀	66	Myomatous uterus
EMW	25	♀	58	Hypertrichosis without endocrine disease
SH	36	♂	60	Primary hyperparathyroidism

analysis of 24-hour specimens, but in some instances the 24 hours were divided into shorter periods For plasma cortisol the Silber-Porter reaction was used as modified by Peterson et al (11) Urinary 17 ketogenic steroids (17-KGS) were determined according to the method of Appleby et al (1) Urinary tetrahydro derivatives of cortisol and cortisone were extracted following beta glucuronidase hydrolysis, purified on silica gel, separated by paper chromatography and determined colorimetrically with blue tetrazolium (12) Total 17 ketosteroids (17 KS) were estimated by the Zimmermann reaction and fractionated 17 KS according to the procedure of Jayle (4) For urinary aldosterone a double labelling isotope dilution technic was applied (8)

Results

As seen in fig 1, both natural and synthetic ACTH induced an increase in plasma cortisol, which was obvious one hour after the commencement of the infusion and then became more pronounced at four and eight hours Four hours after cessation of infusion, plasma cortisol had returned towards normal after tretament with natural as well as synthetic ACTH As judged from the increase in plasma cortisol, the amounts of administered natural and synthetic ACTH, respectively, were about equal with respect to quantitative response

Urinary excretion of 17-KGS showed a similar response following infusion of the two preparations (fig 1) Under the present conditions, no difference could be revealed with respect to the duration of activity of the two preparations In urine specimens collected over four-hour periods, steroid excretion was at its highest level during the last four hours of the infusion and the next four hours following termination of the infusion whereafter the values came down towards normal on the day after the infusion (table II and fig 1) Furthermore the changes induced with respect to urinary excretion of various tetrahydro derivatives (THE, THF and THB) were of the same order of magnitude

duction of both glucocorticoids and androgens and also aldosterone Similar results for the influence of beta[1 24] corticotropin on glucocorticoid production have been reported by Jenny et al (5), Walser and Koller (14) and Karl (7) Jenny et al (5) also noted sodium retention and loss of potassium following the administration of synthetic ACTH, which one could interpret as indicating increased secretion of aldosterone An increased excretion of dehydroepiandrosterone, androsterone and etiocholanolone after infusion of beta[1 24] corticotropin has been reported by Karl (7)

In their studies Walser and Koller (14) found some indication that beta[1 24] corticotropin initiates steroidogenesis faster than natural ACTH and that the effect of the synthetic preparation vanishes sooner than that of natural ACTH Our results seem not to indicate such a difference but further investigations are evidently needed to clarify this point

Our studies as well as those of others support the view that beta[1 4] corticotropin possesses steroidogenic properties identical with or very similar to those of natural ACTH Accordingly this synthetic preparation can be used both for the intravenous ACTH test in patients suspect for adrenocortical disease and for intravenous administration therapeutically This is of particular importance in patients suffering from allergic reactions following the administration of available preparations of natural ACTH there being apparently no such manifestations after beta[1 24] corticotropin (5)

Summary

The steroidogenic properties of synthetic beta[1 24] corticotropin have been investigated following an 8-hour infusion in nine patients It produces increases in plasma cortisol and in urinary 17 ketogenic steroids, 17 ketosteroids, certain tetrahydro metabolites and aldosterone, qualitatively similar to those observed with natural ACTH infused under the same conditions and in the same subjects

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TABLE III Effect of natural (Nat.) and synthetic (Synth.) ACTH on urinary tetrahydro derivatives of cortisone, cortisol and corticosterone (THE, THF and THB respectively)

Pat		THE (mg/24 h)		THF (mg/24 h)		THB (mg/24 h)	
		Nat	Synth	Nat	Synth	Nat	Synth
ML	Control	7.3	4.9	4.9	2.9	< 0.3	< 0.4
	ACTH	17.0	11.9	17.0	9.5	3.4	2.9
BS	Control	2.8	4.2	1.4	2.7	< 0.2	0.8
	ACTH	11.5	12.9	8.2	12.1	3.3	3.6
EW	Control	5.5	5.2	2.7	3.3	< 0.4	< 0.5
	ACTH	13.1	14.1	10.5	13.1	5.2	7.1
EMW	Control	5.7	2.7	3.2	4.0	1.9	< 0.2
	ACTH	12.2	8.8	13.1	10.1	8.8	6.0

TABLE IV Effect of natural (Nat.) and synthetic (Synth.) ACTH on urinary fractionated 17-ketosteroids of patient EW

	Total 17-KS	DHA	A+E	C
Control	9.1	1.2	2.8	2.3
Nat. ACTH	19.1	5.1	4.8	7.7
Synth. ACTH	17.2	4.9	5.2	5.7

(DHA = dehydroepiandrosterone, A = androsterone, E = etiocholanolone and C = 11-oxygenated compounds)

TABLE V Influence of synthetic ACTH on urinary aldosterone (μg/24 h) under restricted salt intake (less than 10 mEq/day)

Pat	Normal diet	Salt restriction	Salt restriction + synth. ACTH
AGW	4 – 7	17	28
SH	8 – 10	30	48

after synthetic as compared to natural ACTH (table III), supporting the view that the two preparations exerted a *similar influence on the production of* glucocorticoids by the adrenal cortex.

Both preparations induced a marked increase in urinary 17-KS (fig. 1), and on fractionation of 17-KS no qualitative difference could be established (table IV).

In two normal subjects, maintained on a salt-free diet (less than 10 mEq sodium/24 hours) the infusion of beta$^{1-24}$ corticotropin was followed by a clear cut increase in urinary aldosterone (table V).

Discussion

The present studies clearly show that synthetic ACTH consisting of beta$^{1-24}$ corticotropin exerts steroidogenic properties similar to those of natural ACTH[1-3]. The synthetic preparation thus stimulates the adrenocortical pro-

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The Third Department of Medicine, University of Helsinki and the Kivela Hospital, Helsinki, Finland

"Subclavian-steal" Syndrome

By

A. Eisalo, P. Ahos and Esko A. Nikkilä

That an occlusion or stenosis of the subclavian or the innominate artery proximal to the origin of the vertebral artery may cause a reversal of blood flow in the latter was first demonstrated radiologically by Contorni (3). The actual clinical significance of this peculiar circulatory disturbance was generally recognized through the report of Reivich et al (8) who showed that the shunt flow was great enough to cause cerebral ischaemia with neurological symptoms. A number of patients suffering from this new vascular syndrome — appropriately called subclavian steal (4) — have been subsequently described (1, 2, 5, 6, 7, 9, 10). The compensatory blood flow through the brain is usually of sufficient magnitude to prevent brachial ischaemia even on total occlusion of the subclavian artery. During arm exercise the diversion of blood from the brain to the upper extremity increases and cerebral anoxia ensues.

The syndrome seems to be not uncommon. The present report describes 4 cases which were detected in our department within a period of a few months, and in which the collateral circulation occurred through different channels. In one case the retrograde flow was demonstrated to occur along both the vertebral and the common carotid artery, a circulatory pattern not previously described.

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Case reports

Case 1. A 51 year-old stock room man, had had coronary occlusion in 1955. A difference was observed in 1959 in the blood pressures in the right and left arm: left 115/80, right 200/90. In 1960 there had been a brief syncopal attack not associated with peripheral paresis. The patient was admitted because of increasing episodes of vertigo. The attacks had occurred especially in the upright position and had frequently been accompanied by nausea but not vomiting. They had stopped when the patient lay down. There also was a history of symptoms of intermittent claudication of the left leg of 6 months duration brought on by walking some 500 metres. During exercise of the left arm the patient had observed that it became numb more readily than formerly



A B

Fig 2 Case 2 In the injection phase (A) filling of the innominate, subclavian common carotid and vertebral arteries on the right and of the common carotid artery on the left is observed. Two seconds after injection (B) there is seen retrograde filling of the left vertrebral artery evidently by the vertebro-vertebral route and flow of contrast medium into the distal part of the subclavian artery

admitted because of a sudden attack of dizziness and vomiting For about one month there had been occasional attacks of vertigo and the left arm had become tired at work more easily than before During rapid movements of the arm he had noticed numbness in it. During the first two or three days in hospital there was nystagmus but no other objective neurological symptoms Cardiovascular examinations gave no pathological findings other than a systolic bruit over the left subclavian artery and a difference in the brachial blood pressures left side 100/80 right side 140/100 Otherwise physical examination gave normal findings The peripheral blood picture was normal Total cholesterol was 300 mg/100 ml Urine analysis was normal Aortography was performed by passing the catheter through the right femoral artery into the aortic arch During the injection phase (fig 2 A) the innominate subclavian, common carotid and vertebral arteries on the right side appeared normal On the left side a normal common carotid artery was likewise visualized in the injection phase but there was marked stenosis at the base of the subclavian artery and only the base of the artery filled very poorly with contrast medium Two seconds after the injection (fig 2 B) retrograde filling of the left vertebral artery occurred and the contrast medium passed into the distal part of the left subclavian artery

Comment The case was one of left subclavian steal in which the most probable right-to-left communication was vertebro-vertebral Circulatory insufficiency could not be demonstrated in other peripheral arteries However, the patient had suffered previously from coronary occlusion.

Case 3 A 42 year-old carpenter, had a history of spells of dizziness of 5 years duration and of transient episodes of unilateral blindness in the left eye for the past 6 months The latter had increased in frequency occurring 2 or 3 times daily and lasting 5 to 8 minutes. Lately there had been also impairment of vision in the right eye The onset of transient blindness was sudden and was not associated with work. On the other hand the patient had observed that vision was restored if he lay down. Central left facial paresis had also occurred occasionally Physical examination revealed neither cardiac murmurs nor signs of congestive heart failure A weak systolic bruit was heard over the right carotid artery and a strong systolic bruit over the left subclavian artery and both femoral arteries. A normal dorsalis pedis artery pulse was palpable on both sides. Blood pressure in the right arm was 180/85 and in the left arm



Fig 1 Case 1 (A) Injection phase showing filling of the right common carotid and subclavian arteries The left vertebral artery begins to fill 3 seconds after the injection (B) evidently through a communication with the right ascending cervical artery and by the vertebro-vertebral route Aortogram made 5 seconds after injection (C) shows complete proximal occlusion of the subclavian artery and filling of the distal part of the left subclavian artery

The cardiovascular condition was as follows Auscultation of the heart revealed nothing abnormal, and there were no signs of congestive heart failure The carotid artery was palpable bilaterally and no bruits were audible at this site A strong systolic bruit was heard over the left subclavian and the right femoral arteries The posterior tibial artery and the dorsalis pedis artery were not palpable in the left extremity Blood pressure in the right arm was 200/95 and in the left arm 115/75 The physical examination showed no abnormal findings in other organs The haematological status was normal, total serum cholesterol was 280 mg/100 ml The urine did not contain protein or glucose

Retrograde aortography was attempted several times by passing the catheter through the right femoral artery, but the tip of the catheter was stopped by obstruction at the junction of the arch of the aorta and the descending aorta The catheter was therefore passed through the right brachial artery to the innominate artery, where the tip stopped at an obstruction at the level of the second thoracic vertebra Manual injection of 20 ml of 60 % Urografin demonstrated proximal occlusion of the innominate artery During injection (fig 1 A) filling of the right subclavian artery and the common carotid artery was observed but the right vertebral artery was not visualized Three seconds after the injection was begun (fig 1 B) retrograde filling of the vertebral artery and cervical arterial branches occurred on the left side of the neck, the contrast medium then passed into the left subclavian artery and flowed both proximal and distal to the origin of the vertebral artery (fig 1 C) There was complete proximal occlusion of the subclavian artery The communication between the innominate artery and the aortic arch as well as the condition of the left common carotid artery remained unsolved in the examination

Comment In a case of left subclavian steal, communication with the left vertebral artery was most probably maintained through the right ascending cervical artery and by the vertebro vertebral pathway The patient had generalized *obliterating atherosclerosis* clinical manifestations of which were coronary occlusion and symptoms of intermittent claudication of the left leg

Case 2 A foreman aged 44 who had suffered from anterior myocardial infarction in 1962 After recovery from this he has had no anginal pain and is under constant anticoagulant treatment About one year later he was



A B

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Fig 3 Case 4 Injection phase (A) *shows occlusion of the innominate artery* and narrowing of the base of the left common carotid artery Two seconds after injection (B) the right vertebral artery fills by retrograde flow, and 2 seconds later (C) there also is retrograde filling of the right common carotid artery

95/85 No special findings were made in other organs in the physical examination The peripheral blood picture was normal Total serum cholesterol was 330 mg/100 ml The urine was normal Aortography was made by passing the catheter through the right femoral artery At the time of injection the right innominate, subclavian, common carotid and *vertebral arteries and the left common carotid* artery were visualized An annular stenosis was seen at about the middle of the innominate artery The right common carotid artery was greatly narrowed over a length of about 2 cm at the level of the 4th and 5th cervical vertebrae The right vertebral artery was dilated The left common carotid artery was narrowed throughout its length and the wall was uneven Three seconds after the injection retrograde filling was seen in the left vertebral artery *and the contrast medium* was flowing into the distal part of the subclavian artery Bilateral carotid angiography *disclosed marked narrowing of the common* carotid artery and of the origin of the external and internal carotid arteries on the right side On the left side of the neck were seen arterial branches that had filled by way of a collateral circulation through the right superior thyroid artery The left carotid angiogram showed narrowings at the origin of both the internal and the external carotid artery The patient was treated by bypass operation and is at this time symptom free

Comment Left subclavian steal, with collateral circulation to the left vertebral *artery through the right superior thyroid* artery Both carotid arteries and both femoral arteries showed obliterative atherosclerosis

Case 4 A 51 *year old married woman*, had a history of bronchial asthma of 17 years' duration During the past 8 years she had observed numbness accompanied by slight cyanosis in the right arm and hand on working No neurological symptoms were present When re-admitted because of increasingly severe dyspnoeic episodes whistling dry râles were heard over the lungs but without signs of congestive heart failure A systolic bruit was clearly audible over the left carotid and subclavian arteries and the right carotid artery The peripheral arteries of the lower extremities appeared normal Blood pressure *was not measurable in the right arm* and 90/75 in the left arm The peripheral blood picture was normal with the exception of occasional mild eosinophilia Total *serum cholesterol* was 310 mg /100 ml The eyegrounds were normal Urine analysis was normal In aortography the catheter was introduced through the right femoral artery to the aortic arch During the injection phase (fig 3 A) complete occlusion of the origin of the innominate artery was observed The origin of the

left common carotid artery was narrowed over a length of 2 cm and in this area the wall was uneven The left subclavian artery showed narrowing and roughness of the wall proximal to the origin of the vertebral artery The left vertebral artery was 5 mm in diameter and had smooth walls At 2 seconds after injection (fig 3 B) retrograde filling of the right vertebral artery was seen and at 4 seconds (fig 3 C) the right common carotid artery was also visualized by retrograde flow Contrast medium passed from both arteries to the right subclavian artery

Comment Right subclavian steal, in which the right subclavian artery was supplied by retrograde flow from both the right vertebral and the right common carotid artery The right vertebral artery most probably had a vertebro-vertebral communication to the left side, and blood from the right common carotid artery was most probably channelled to the left carotid circulation through the anterior communicating artery

Discussion

The neurological symptoms associated with the subclavian steal syndrome are considered to be a result of basilar artery insufficiency The latter, in turn is most probably related chiefly to three causes *1* the volume of retrograde blood flow 2 the anatomical condition of the afferent cerebral arteries, and 3 the non cerebral collateral channels The blood volume diverted from the basilar circulation to the retrograde circulation is probably not the sole conclusive factor in the patient s symptoms A good example of this is case 4, in which the retrograde flow from the left side to the distal part of the right subclavian artery was channelled through both the vertebral and the common carotid artery A considerable blood volume was thus removed from the cerebral circulation Nevertheless the patient at no time had neurological symptoms but only claudication of the right arm A tentative explanation for this may be that the retrograde flow in the right vertebral artery is too small to cause basilar insufficiency because the main blood supply of the right arm is provided through the right carotid artery However, this patient showed such marked stenosis of the arteries leading to the brain on the left side that the blood supply to the brain was very evidently smaller than normal Under such circumstances even a slight retrograde flow should give rise to neurological symptoms, particularly since compensatory flow through the right carotid artery which usually occurs in cases of subclavian steal (8), was not possible The only explanation for the absence of neurological symptoms is possibly a strong collateral circulation Methods so far available do not permit estimation of the capacity of the collateral channels available for the cerebral blood supply Only the large collaterals are visualized in angiography The findings obtained by postmortem injection methods hardly provide a *true picture* of the collateral network of the *brain of* a living subject The formation of a system of collaterals in slowly *progressing* atherosclersosis is *undoubtedly subject* to considerable individual variation governed in part by the site of the atherosclerosis and *in part by* the original anatomical variations

Why are symptoms from the brain more prominent than *those from* the



A B C

Fig. 3. Case 4. Injection phase (A) shows occlusion of the innominate artery and narrowing of the base of the left common carotid artery. Two seconds after injection (B) the right vertebral artery fills by retrograde flow, and 2 seconds later (C) there also is retrograde filling of the right common carotid artery.

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From the Department of Internal Medicine the Harbour Hospital Institute of Tropical Diseases, Rotterdam Leiden and the Central Pathological Laboratories, Municipal Hospitals Rotterdam, Holland

Steatorrhoea and Ulcerative Jejuno-ileitis

By

H Smitskamp and F C Kuipers

When a patient is seen with severe steatorrhoea and no specific cause can be detected, a diagnosis of ideopathic steatorrhoea, or non tropical sprue, is readily made The diagnosis is usually supported by the prompt response to gluten free diet or spontaneous remission Steatorrhoea and diarrhoea per se are seldom fatal, and these patients can be kept in a fairly normal state of health for long periods until intercurrent infections cause a fatal termination

Recently, however, a few observations have been recorded of previously undescribed clinical and post mortem findings in patients with steatorrhoea In 1949 Nyman, in 1952 Faber et al, and in 1957 Himes et al described patients with severe steatorrhoea and a fatal outcome The autopsy showed a marked ulceration of the small intestine quite different from the picture usually seen in non tropical sprue or in regional ileitis

A more or less identical case is presented here Himes et al discussed the possible bearing of corticosteroid therapy on the fatal outcome in their patient In contrast with their patient who received these hormones during a period of 2 1/2 years the corticosteroids were given to our patient only during a short period at the end of the illness The patients of Nyman and Faber et al did not receive this therapy at all We are unable to include these cases in any known clinical or pathological entity

Submitted for publication June 2 1964

Case report

A 36 year-old Norwegian woman who travelled with her husband on board a tanker, was admitted to the Hospital on May 12, 1960 She had *become acutely ill* one week previously Medical treatment on board the ship had not been possible

The patient appeared seriously ill and very anaemic She was drowsy, and a complete *history could* not be obtained Her chief complaint was *frequent bowel* movements during the past week The stools had been clay-coloured and fatty initially but had shown a dark red colour for the last 3 days and there had been a rapid increase in general lassitude and weakness

arm, although it would seem that the ischaemia is greater in the arm, at least in a case of total occlusion? The answer is probably that the brain is much more sensitive to anoxia Or possibly in the patients with neurological symptoms the cerebral circulation is already impaired and the brain at the border of anoxia, a small decrease in the blood flow gives rise to immediate symptoms

Though we are not yet able to analyze completely the part played in the cerebral circulation by a proximal occlusion of the subclavian artery, practical clinical work has taught us that by vascular surgery we can greatly relieve the subjective neurological symptoms of the patient suffering from the subclavian steal syndrome (6, 8) Since the syndrome has only recently been presented, there is no definite information on its incidence However, in view of the high incidence of atherosclerosis it would seem likely that the subclavian-steal syndrome is relatively common When a proximal occlusion of the subclavian artery is suspected, examination will be facilitated by a few simple orientative measures arterial auscultation and palpation on the neck and over the clavical, and measurement of the blood pressure in both arms A definite diagnosis is possible only after aortography, which will localize the occlusion, show the patency of the other arteries rising from the aortic arch, visualize the areas of collateral circulation, and reveal the retrograde flow

Summary

Four cases of subclavian steal syndrome are described In each case the collateral circulation communicating with the retrograde flow in the vertebral artery occurred through different channels In one case the retrograde flow to the distal part of the subclavian artery occurred simultaneously along both the vertebral and the carotid arteries

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total protein 46 %, albumin 19 % and a reversed albumin/globulin ratio

The liver function tests were within normal limits X ray examination of the intestinal tract showed some segmented and dilated jejunal loops but was otherwise normal

Treatment consisted of a high caloric and gluten free diet and the administration of folic acid, 15 mg per day This resulted in a prompt haematological response with reticulocyte crisis up to 48 ‰ and the passage of formed stools once or twice a day The patient gained weight rapidly The patient was discharged and remained free of symptoms until Jan 1961, when she was re admitted with a sudden recurrence of abdominal cramps and diarrhoea She had developed subfebrile temperatures and examination showed general distress and a diffusely tender abdomen The stools were copious contained blood as before some mucus and large amounts of fat The fat balance studies and D xylose tests gave about the same results as during the previous ad mission Again the plasma prothrombin time was 10 % (one-stage Quick method) and the haemoglobin level was down to 10 8 g % The intestinal bleeding stopped and the plasma prothrombin time became normal immediately after administration of Vitamin K_1

However in spite of strict gluten free diet and folic acid administration there was no improvement The diarrhoea persisted be came even more severe and the general condi tion deteriorated rapidly

The temperature became higher up to 39 C Repeated stool cultures revealed no pathogenic micro-organisms A cause for the fever could not be established In order to sterilise the intestinal contents 1 g per day of a broad spectrum antibiotic achromycin was given without any results A blood cul ture now revealed streptococci of several types streptococcus hominis and strepto coccus salivarius sensitive to chloromycetin The administration of this antibiotic resulted in gradual return of the temperature to nor mal It had no influence on the diarrhoea which became more profuse and more frequent every day Notwithstanding the fact that the patient ate practically nothing due to very poor appetite and psychic depression, the stools continued to contain large amounts of fat Dehydration set in and intravenous administration of fluids via a polyethylene catheter in the vena cava became necessary As an ultimate measure corticosteroids were given in fairly high doses (4 8 mg dexame thason per day) from Feb 15 until the day before death on Feb 28 The total amount of liquid stools rose to 3—4 000 cm³ per day and fluid and electrolyte balance was main tained with difficulty On Feb 27 the patient suddenly complained of severe abdominal pain The abdomen became distended peristalsis disappeared and board like rigidity developed On Feb 29 the patient died with the final diagnosis of perforation peritonitis

Post mortem report

At autopsy 11 hours post mortem the body appeared cachectic the subcutaneous adipose tissue on the chest measured 0 3 cm on the abdomen 0 5 cm There was a scar of a median laparotomy in the upper abdomen. The peritoneal cavity contained about 1,600 ml cloudy fluid with fibrin, the peri toneal surface was dull red The pleural and pericardial cavity were obliterated by firm adhesions The heart was small without abnormalities the lungs were oedematous at the bases and had dilated bronchi in the basal segments In the intestinal tract the jejunum and ileum were seriously altered the wall was thickened in many parts sometimes oedematous and firm greyish red in other areas The mucosa was also oedematous with considerable ulceration of its surface The ulcers did not appear to be localised and were often transversely arranged The ulcers measured from 0 3 by 1 cm up to 0 7 by 2 cm About 30 cm proximal to the ileocoecal valve one of the ulcers was perforated The contents of the small intestine were a yellow brown fluid The large intestine had the same contents but the mucosa and the muscle wall appeared normal to the naked eye There was a sharp border at the ileocoecal valve between the diseased ileum and the

The temperature on admission was 38° C, pulse rate 120/min, blood pressure 90/65 There was incontinence of large amounts of *dark-coloured, foul smelling stools* The patient was extremely weak and thin There was no abnormal skin pigmentation The tongue showed some atrophy of the papillae The heart and lungs appeared normal The abdomen was not distended, the bowel sounds were increased The liver and spleen were not enlarged A trace of pre-sacral oedema was present Neurological examination was normal

The haemoglobin was 5 9 g %, erythrocytes 2,090,000/mm³, the leucocytes 5,100/mm³ The differential count of the white cells was normal with anisocytosis, poikilocytosis and polychromasia of the red cells Thrombocytes 230,000/mm³ Bleeding time 1'30", clotting time 3 30", prothrombin time (one-stage Quick method) 9 % Urine *analysis showed results within normal limits* The stools contained numerous fat droplets and fatty acids, but the main substance was pure blood

The first two days the patient received 2,500 ml of blood The administration of 10 mg vitamin K_1 (mephyton) intravenously resulted in a prompt rise of the prothrombin level to 90 %, and the intestinal bleeding stopped completely after two days

A more extensive history now revealed that the patient *remembered having frequent* bouts of diarrhoea during childhood In 1940—1941 she had pleuritis and pericarditis No active diseases had been detected at regular chest X rays since then In 1951 she had the first recurrence of abdominal complaints there being a period of about 4—5 months of pain in the epigastric region with frequent bouts of diarrhoea At that time she was not treated in a hospital In 1958 she had a sudden onset of diarrhoea, colic, epigastric pain and weight loss and was hospitalised in Stavanger in Norway The following observations were made there

Blood cultures stool cultures Widal and Bang tests gave no clue to the nature of the disease sometimes blood was found in the stools The X ray examination suggested a stricture or tumour in the distal part of the duodenum The colon appeared normal

A laparotomy was performed The only abnormality found was a mild oedema of the wall of the small intestine and enlargement of the mesenteric lymph nodes Pathological anatomical examination of the specimen removed showed simple reactive changes in a normal lymph node

Post operative recovery was prompt and she was able to leave the *hospital after two* months, in fairly good condition, having normal bowel movements and no abdominal pain, moreover she was gaining weight

She had a symptom free interval until Dec 1959 when the abdominal complaints returned, again anorexia, abdominal cramps diarrhoea and rapid weight loss Conservative treatment resulted in partial recovery and in April 1960 she joined her husband on a sea voyage A recurrence of symptoms *seven days prior to our seeing her, resulted* in her admission here in Rotterdam

The initial diagnosis of idiopathic steatorrhoea was made, possibly connected with coeliac disease in childhood, with a bleeding tendency due to poor vitamin K absorption Further laboratory investigations gave the following results

Fat balance studies on a standard diet, containing 50 g of fat, revealed an average excretion of 43 g fatty acids and 8 g neutral fats per 24 hours Two months later, in July 1960 when the diarrhoea had stopped approximately the same values were obtained (33 g fatty acids 7 g neutral fat per 24 hours)

The D xylose *tolerance test showed* a D xylose blood level 2 hours after oral ingestion of 25 g D xylose, of 13 7 mg % (normal values in our laboratory 20—70 mg %) In the five-hour urine specimen (*altogether*) 2 4 g D xylose was found (normal values 4 1—8 2 g) The anaemia was of a slightly hyperchromic type M C V 112 μ³— M C H 32γ M C H C 30 %

The sternal marrow showed mild erythroblastic hyperactivity no megaloblasts were seen

Serum calcium 8 4 mg % serum phosphorus 3 4 mg % The serum proteins were low

organism, also because of the finding in the blood culture of a streptococcus hominis and streptococcus salivarius These bacteria, however could not be cultured from the stools, and the administration of oral antibiotics to which the micro-organisms were sensitive had no influence on the diarrhoea

3 The resistance to any form of therapy in the final stage makes it extremely doubtful whether the response to gluten free diet and folic acid during the first admission was actually due to this treatment It might have been a spontaneous remission just as had occurred previously with simple symptomatic therapy It is difficult to evaluate the influence of the corticosteroids which had been administered in the last stage It might be wise to withhold this form of therapy in patients with steatorrhoea who have a progressive watery diarrhoea We think that the extensive small bowel ulceration had nothing to do with the corticosteroid therapy and represents a disease by itself which it is impossible to classify However the idea that the hormone administration had a direct bearing on the ultimate perforation of the small ulcers is not too far fetched

From a clinical point of view there is a striking similarity *between our* patient and the patient described by Himes et al Also the reports from *Faber et al and* Nyman show similar clinical courses

At post mortem however the findings were somewhat different from those of the above mentioned authors The patient of Himes et al had almost completely hyalinised mesenteric lymph nodes with dilated sinuses containing sudanophilic material, whereas our patient had unspecific reactive changes in these nodes three years before death as well as at post mortem

Faber's patient had an infiltration of mononuclear cells only in the pancreas and the duodenum, while in our patient the pancreas was normal and the infiltration was pronounced over the whole length of the small intestine

Only the case described by Nyman had the same clinical and pathological features as our patient, although the cause of death was cachexia and bronchopneumonia and no perforation of any of the ulcers was found An explorative laparotomy with jejunal biopsy was done previously and revealed the same histological picture as at post mortem It is interesting that in Nyman s case at laparotomy the inflammatory reaction was confined to the jejunum and the proximal part of the ileum, whereas at post mortem it extended much farther down the ileum *In our case at the time* of explorative laparotomy *there was* radiographic evidence of a *possible* obstruction in the duodenum, and *the* surgical report mentioned only oedema of *the small intestine* At post mortem however the whole small intestine was diseased, a superficial cellular infiltration was present in the colon also This may be an *indication* that the disease started in the proximal part of the small intestine and in course of time spread to the more distal parts as a continuous process This is *not usually* seen in regional enteritis, moreover *tuberculoid* formations were *not found* anywhere in the intestine or lymph nodes It therefore seems incorrect *to* diagnose this disease as classical or

apparently normal colon The mesenteric lymph nodes were swollen up to 2 cm diameter, grey red and moist on the cut surface The liver was soft and had a pale yellow-red colour The other internal organs were normal The spleen weighed 160 gram and appeared normal

Microspical examination of jejunum and ileum revealed a heavy infiltration of mononuclear cells in the wall The infiltrate consisted of swollen reticular cells, plasma cells and a few eosinophilic leucocytes, and extended from the mucosa to the serosal surface Polynuclear leucocytes were not seen, except at the margins of the perforated ulcer There were many ulcers elsewhere, occasionally extending deeply into the muscular layer The mucosa was atrophic but lesions specific for steatorrhoea could not be discerned, as the tissue was already slightly autolysed Vascular necrosis was not observed The mesenteric lymph nodes had small follicles and wide sinuses, crowded with histiocytes, plasma cells and a few eosinophilic leucocytes There was no fibrosis, nor could lipid be demonstrated In the colonic mucosa a moderately heavy infiltrate of the same cells as in the ileum was present, but no ulceration

The muscle wall of the large intestine was entirely free of infiltration Culture of the contents of the intestines for tubercle bacilli was negative

In the red pulp of the spleen many swollen reticular cells, plasma cells and a few megakaryocytes were present, otherwise the spleen was normal The liver showed conspicuous fatty infiltration In kidneys, adrenals, pancreas, thyroid, trachea, heart and lungs no histological abnormalities were present

Discussion

This patient presents several unusual features that differ considerably from those seen in uncomplicated idiopathic steatorrhoea

1 The abundant intestinal bleeding seen at the first admission had been present previously to a minor degree and also during the second admission In view of the low plasma prothrombin level and the prompt response to parenteral administration of vitamin K_1 in both instances, it can be assumed that hypoprothrombinaemia due to impaired intestinal absorption of vitamin K_1 was the main causative factor In idiopathic steatorrhoea, however, the usual manifestations of hypoprothrombinaemia are haemorrhages in the skin and bleeding from nasal and oral mucous membranes These were not present in this patient at any time during the periods of observation

Presumably the alterations in the intestinal mucosa predisposed to excessive haemorrhage in this area before bleeding of the skin or other mucous membranes could occur

Himes et al suggest that in their patient the bleeding potential was aggravated by the use of corticosteroids It should be noted that in our patient the bleeding periods occurred before any such therapy was given

2 Faulty fat absorption often causes production of copious foul smelling stools of a semi liquid character Sometimes, especially when the amount of unabsorbed fat in the stool is not too high, normally formed stools can be present The large amounts of watery diarrhoea observed during the final period in this patient are generally not found in idiopathic steatorrhoea, and indicate a different pathological process

We have considered the invasion of the small intestine by a pathological micro-

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A Case of α_2-Myelomatosis

By

E Sotaniemi, T Niinimaki, O Rasänen and T Vartio

Wuhrmann and Wunderly (7) were the first to classify the myelomas on the basis of the electrophoretic distribution of serum globulins into groups exhibiting pathological fractions in the alpha, beta, beta gamma and gamma regions and other groups having a normal electrophoretic pattern (6)

Myeloma cannot, however, be diagnosed by electrophoresis alone Before one can speak of a myeloma the so-called myeloma cell must be found, while observations of the clinical picture, and X ray and laboratory examinations are also of importance

α_2 myelomas are unusual, and only isolated cases are described in the literature According to Tidstrom (4) absolutely certain cases of α_2 myeloma *have been reported by Haarstad et al* (1) one case Wiedemann (6), one case, Wuhrmann and Wunderly (7) two cases Laurell (3) one case, Jansen (2), one case and Tidstrom himself one case, a total of 7 cases Numerous other cases of α_2 myeloma have been described, but their diagnosis has been based solely on the α_2 peak visible on serum electrophoresis This peak may be produced by myelomas but is also produced by infections and generalized carcinomatosis ' (5)

One characteristic of patients with α_2-meyloma has usually, though not invariably, been a rapid deterioration of general condition, together with an abundance of immature cells, many of them myelomatous forms of plasma cell leukemia type, in sternal marrow The ESR has usually exceeded 100 mm/h and serum electrophoresis has shown, besides the raised α_2 peak, a low or absent γ peak

Case report

General laborer aged 60 years With the exception of pleurisy (non tuberculous) at the age of 30 he had always been healthy In Feb 1963 the patient hurt his hip while doing forest work and began to limp with his right leg At the same time his general condition deteriorated rapidly Subjectively

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atypical regional enteritis It seems reasonable to consider the case presented, as well as Nyman's case, as an unusual ulcerative jejuno-ileitis, clinically manifesting itself as idiopathic steatorrhoea The patient described by *Himes et al* may have had the same disease, but in a later stage, with cicatrizing of previously inflamed mesenteric lymph nodes

Summary

A patient has been described who presented clinically and initially a picture of idiopathic steatorrhoea with severe intestinal bleeding due to vitamin K deficiency

Finally an uncontrollable watery diarrhoea developed, and the patient died of perforation peritonitis after having received corticosteroids The autopsy revealed an extensive ulcerative process in the small intestine with a perforation Such findings have seldom been reported and defy classification

Acknowledgement

We are indebted to G Blikre, M D, Stavanger sykehus, Stavanger, Norway

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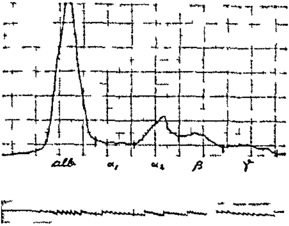


Fig 1 Serum paper electrophoresis

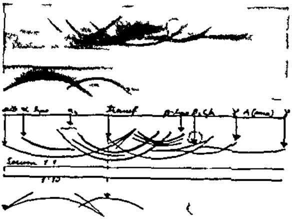


Fig 2 Serum immunoelectrophoresis

the patient had suffered most from pain in the right hip weakness and fatigue

General examination (April 16 1963) The patient was small in size and of slender build and in poor condition and emaciated (height 152 5 cm weight 41 0 kg) During the examination he was too weak to sit up for long His skin was dry and lacked elasticity The subcutaneous tissue had virtually disappeared

X ray examination All over the bones of the skull thorax lumbar spine and hip there were circular clear spots The size and shape of the heart were within normal limits The lungs were emphysematous and a striated infiltrate was visible below the left hilus

Bone marrow Erythropoiesis was scant normoblastic Pronounced anisocytosis Granulocytopoiesis was scant The specimen contained large quantities of lymphocytes. The majority of cells were mature plasma cells and plasmacellular reticular cells Thrombocytopoiesis was normal Diagnosis Myelomatosis (Totterman G , M D)

Laboratory tests

1 *Blood*

ESR 105 mm/h AST 100 ASTA 2 0 Blood picture Hb 9 4 g/100 ml RBC 2 15 million MCH 38 aniso and poikilocytosis WBC 23,000 Differential count neutrophils non segm 2 0 segm 22 0 eos 0, bas 0 mon 2 5 lymph 11 0 myelocytes 0 5 % Cells having the appearance of plasma cells 52 % of myeloblasts 10 0 % of normoblasts *1/200 WBC Thrombocytes 250 000*

Blood chemistry alkaline phosphatase 28 B L units acid phosphatase 0 65 B L units serum calcium 13 4 mg/100 ml serum P 2 45 mg/100 ml creatinine 1 25 mg/100 ml serum iron 188 μg/100 ml

Serum electrophoresis (paper fig 1) albumin 56 7, α 7 7, α_2 16 6 β 11 7, γ 7 5 relat % total protein 6 0 g/100 ml

Serum immunoelectrophoresis (fig 2) true γ globulin (weak) no observable γ_1 A globulin but β_2 C globulin markedly increased and of myelomatous type Other proteins normal (Vahvaselka E M Sc State Serum Institute)

Ultracentrifugation of serum

	S	%
Albumin	4 9	88 4
Globulin	7 0	9 9
Macroglobulin	19 6	1 7

Ultracentrifugation of serum was normal but this result does not preclude a myeloma since the pathological fraction may travel with the albumin fraction (Seppälä P M D Institute of Medical Chemistry Turku)

2 *Urine*

Protein initially negative later positive Bence-Jones protein negative Sediment macroscopically profuse microscopy revealed a few leukocytes and large quantities of phosphate sediment Bacterial staining and culture negative

Urine electrophoresis (fig 3) albumin 14 1 α-globulin 15 4, α_2 32 4 β 24 9 γ 12 8 relat % Total protein 0 25 %

Urine immunoelectrophoresis (fig 4) urine 50 × concentrated showed albumin α_2 globulin (weak) γ_1 A globulin of the myelomatous type but weak, and traces of transferrin

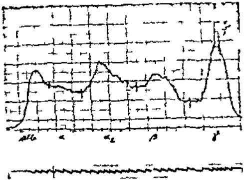


Fig 3 Urine paper electrophoresis

3 *Spinal fluid*

Paper electrophoresis (fig 5) Pre albumin 2 3 albumin 55 9 α globulin 7 0 α_2-globulin 11 6 β 11 6 γ 11 6 relat %

Immunoelectrophoresis spinal fluid 50 × concentrated showed only scant albumin

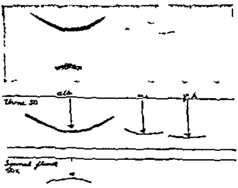


Fig 4 Immunoelectrophoresis of urine (*top*) and of spinal fluid (bottom)

4 *Feces* No occult bleeding

The patient s general condition deteriorated steadily despite drug therapy (Urethane Decadurabolin blood transfusions analgesics)

The patient was unable to move in the ward and finally only able to sit if supported by nurses His weight dropped to 38 5 kg ESR remained throughout at about 100 mm/h Hemoglobin decreased as did the leukocyte count (initially 23 000) to 4 500 The patient died on July 1 1963

Autopsy

Subcutaneous tissue completely lacking bones osteoporotic The calvarium showed greyish red soft foci the sternum grey red foci The intestines revealed no special findings and nothing indicative of neoplastic growth

Microscopic preparation of the sternum The bone marrow contained numerous large slightly acidophilic well-defined cells with an oval nucleus rich in chromatin. The nuclear margins showed spots of chromatin accumulations Many cells had a distinct nucleolus many were undoubtedly similar to the myeloma cell while the cytoplasm of many cells of rather mature appearance contained numerous yellowish granules Diagnosis Myelomatosis (Dammert K M D)

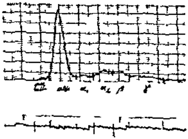


Fig 5 Paper electrophoresis of the spinal fluid

Summary

The diagnosis of α_2 myeloma in the present case is based on observation of the clinical condition for 3 months in the ward on X ray and bone marrow

examinations, serum electrophoresis and immunoelectrophoresis, and ultra-centrifugation of the serum The diagnosis of myeloma was corroborated by post-mortem findings

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Long-term Anticoagulant Therapy of Coronary Disease

A Retrospective Study from an Out patient Clinic 1951—1963

By

PER LUND JOHANSEN[1]

This report seeks to survey the experiences from the first decade of long term anticoagulant treatment in patients with coronary disease at one of the largest out patient clinics in Norway outside Oslo This clinic, The Medical Out patient Department Vestfold Central Hospital, served most of the rural and urban population in Vestfold County and the treatment has been carried out by a changing staff of doctors This report is presented because most reports on anticoagulant therapy at least in this country originate from centers where one or a few experts have guided the therapy and the possibility of maintaining this therapy properly outside such centers has been questioned

Material

All patients (308) who were put on long term anticoagulant treatment for coronary heart disease in the period Jan 1st 1951 to July 1st 1961 and who survived for more than 8 weeks after starting treatment are included in the survey The survey was completed on Jan 1st 1963

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The data were collected from hospital and out patient department records from general practitioners health service authorities questionnaires and personal contact

Almost all the patients started the therapy in the Medical ward at the Vestfold Central Hospital and were followed at the out patient clinic for the whole observation period For geographical reasons 22 patients were transferred to other out patient departments during the observation period, but since detailed information about these patients was available they have been included Only 8 patients were excluded from the survey 3 had moved and were not located and 5 had heart failure or uremia on starting treatment and died within a few months For the remaining 300 patients *the mean observation time* was 3 2 years range 1 1/2 to 12 years and totalling 990 years

The *indications for starting treatment* have changed little during the survey During recent years, however patients above 70 years of age or with mild hypertension have rarely been treated Apart from this and from a doubtful diagnosis and the usually accepted contraindications to anticoagulant therapy (2, 16) *all* patients with myocardial infarction and intermediary coronary

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TABLE I Indications for starting anticoagulant therapy. Numbers in brackets refer to patients with intermediary coronary syndrome. The patients starting therapy on account of recent myocardial infarction are in the following termed the "Infarct group", the rest the "Angina pectoris group"

	♂	♀	Total
Recent myocardial infarction	196	21	217
Intermediary coronary syndrome or angina pectoris	69 (9)	14 (1)	83 (10)

TABLE II Age and sex distribution. Numbers in brackets refer to patients who died

Age groups	Infarct group ♂	Infarct group ♀	Angina pectoris group ♂	Angina pectoris group ♀
24—29	1	0	0	0
30—39	2	0	4	1
40—49	33	0	10	2
50—59	77	3	28	3
60—69	68	15	22	8
70—75	15	3	5	0
Total	196 (55)	21 (5)	69 (23)	14 (1)

TABLE III Previous coronary disease. (Since some patients had both previous infarction and angina pectoris > 1 year, the total sum is more than 100%)

	Infarct group	Angina pectoris group
Previous infarction	21 (10%)	14 (17%)
Angina pectoris > 1 year	49 (23%)	34 (41%)
No previous coronary disease	161 (74%)	38 (46%)

TABLE IV Percentage distribution of all the PP (TT) values from 40 patients randomly selected and of the values in connection with recurrences in cases where data were available (57 episodes)

PP value range (%)	Distribution of PP (TT) values (%): All values from 40 patients randomly selected	In 57 episodes of recurrent infarction (verified and probable)
0—14	34.4	35.1
15—25	41.9	42.1
>25	23.7	22.8

syndrome were treated, as well as an increasing number of patients with angina pectoris. Tables I, II and III give further data on the patients.

Treatment and control of patients

The patients attended the out-patient department for both clinical and laboratory control. They were not given any dietary restrictions but were advised to avoid overweight and excessive smoking. Nitroglycerin was given freely, and sedatives when necessary.

The main anticoagulant drug was phenylindanedione, in the first few years dicoumarol.

The laboratory control was very reliable, the same technician doing all the coagulation tests throughout the entire period. Quick's method was used until 1958, Owren's PP method from then until 1960 and Owren's thrombotest method (TT method) after 1960. The interval between controls was 3—6 weeks at the beginning of the observation period and usually 8—10 weeks in the later years.

TABLE V Survey of maintenance of therapy

	Infarct group	Ang na pectoris group
Cont nuous therapy	183	70
Therapy interrupted	34	13
a) Stopped permanently	29	10
b) Therapy resumed because of infarct on (all died within 8 weeks)	1	3
c) Long term treatment re sumed because of infarct on (2) or suspected infarction (2)	4	0

TABLE VI Causes of cessation of therapy

	Infarct group	Angina pectoris group
Intercurrent d sease	9	3
Lack of cooperat on	6	3
Bleeding	4	4
Pat ent s work (seamen)	6	1
Allergy	4	1
Old age	4	0
Stopped by other phys c an	1	1
Total	34	13

Cancer 5 cyst c k dneys 2 and 1 of each of the fo lowing diseases hepat tis urol thias s amyo trophic lateral sclerosis dementia valvular heart d sease

TABLE VII Infarctions during treatment

	Infarct group	Angina pector s group
No of pat	217	83
Pat ent months of exposure	7 338	3 468
Total no of infarcts	66	22
a) Verified	36	12
b) Probable	30	10
Total no of infarcts per 100 patient months of exposure	0 90	0 63

TABLE VIII Deaths dur ng treatment The non cardiac deaths were cerebral hem orrhage 2 cerebral thrombosis 1 and d ssecting aneurism of aorta 1

	Infarct group	Ang na pector s group
Deaths from ver fied infarcts	18	6
Deaths from probable infarcts	25	9
Deaths from cardiac fa lure	5	0
Total no of card ac deaths	48	15
Card ac deaths per 100 patient months	0 65	0 43
Total no of non-cardiac deaths	3	1

Intens ty of the anticoagulant therapy

During the period when Qu ck s method was used a sample taken at random showed that 64 % of the values were in the range of 2—2 1 2 t mes the normal prothromb n t me the majority of the rest be ng shorter S nce the Quick method is no longer used here and s nce most of the patients have been controlled by Owren s meth ods a detailed study of the intensity of treatment is confined to the period when these were in use The aim was to keep the PP (TT) values between 10 % and 25 % s nce 1960 between 10 % and 20 % (5) The *average value* for 40 pa t ents randomly selected (781 tests) was 18 2 % The distribution of the values is seen from table IV which also shows the distr but on of the values in connection with

TABLE IX. Infarctions during period without treatment

	Infarct group	Angina pectoris group
No. of pat.	34	13
Patient months of exposure	648	432
Total no. of infarcts	6	6
a) Verified	4	2
b) Probable	2	4
Total no. of infarcts per 100 patient months of exposure	0.93	1.4

TABLE X. Deaths during period without treatment. The non cardiac causes of death were: cancer 4, amyotrophic lateral sclerosis 1.

	Infarct group	Angina pectoris group
Verified infarcts	2	2
Probable infarcts	2	4
Cardiac failure	1	1
Total no. of cardiac deaths	5	7
Cardiac deaths/100 patient months	0.77	1.6
Total no. of non cardiac deaths	4	1

57 episodes of verified or probable recurrent myocardial infarction. It is seen that the distribution is the same as in the total period.

No differences were found between surviving patients and patients who died of myocardial infarction regarding the average value or the distribution of the PP (TT) values.

Duration of therapy

Anticoagulant therapy was not discontinued unless certain circumstances made it necessary. This happened in 47 patients (table V) for reasons evident from table VI. Except in cases of hemorrhage, the treatment was discontinued gradually over 1—2 weeks. Seamen, who stopped treatment in order to go back to sea, were treated for at least 3 months.

Further details regarding the duration of therapy are shown in tables XI and XII.

In 1962 discontinuance of treatment was discussed in symptom free infarct patients treated for several years, but when suggested to the patients it caused great emotional reactions and the project had to be abandoned. Similar observations have been made at other clinics in the county.

Results

The criteria of Harvald et al. (10) have been followed in classifying infarctions and causes of death. Data for *infarctions* and *deaths* during the treatment period are given in tables VII and VIII, and during the period without anticoagulant therapy *in tables* IX and X.

The *survival rate* is calculated according to the actuarial method (22) for the *period with treatment* (tables XI and XII) (for the time without treatment the numbers are too small).

Fig. 1 shows the survival of the infarct group in this and some other Scandinavian surveys (2, 10, 14, 16). Their comparability is discussed later. Figs. 2 a and b *show the survival of the infarct* patients according to age in this and 3 other Norwegian surveys (2, 16, 20).

Fig. 3 shows the survival of the angina pectoris group in this and 2 other Norwegian surveys (19, 22) and fig. 4 the survival rate according to duration of

TABLE XI Calculation of survival rate for patients in the infarction group during treatment

Period of exposure (year)	No of pat under treatment at end of period	No of deaths	Observation discontinued: Treatment stopped	Observation discontinued: Observation ended	Exposure man year	Probability of dying in interval	Survival rate
Start	217	—	—	—	—	—	100
1	184	16	17	0	208 5	0 0767	92 3
2	145	7	9	23	168	0 0417	88 5
3	100	8	6	31	126 5	0 0632	82 9
4	70	6	2	22	88	0 0617	77 8
5	43	9	0	18	61	0 1467	66 3
6	22	4	0	17	34 5	0 1159	—

TABLE XII Calculation of survival rate for patients in the angina pectoris group during treatment

Period of exposure (year)	No of pat under treatment at end of period	No of deaths	Observation discontinued: Treatment stopped	Observation discontinued: Observation ended	Exposure man year	Probability of dying in interval	Survival rate
Start	83	—	—	—	—	—	100
1	76	3	4	0	81	0 0370	96 3
2	59	5	7	5	70	0 0714	89 4
3	43	1	1	14	51 5	0 0194	87 7
4	34	2	1	6	40	0 0500	83 3
5	22	3	0	9	29 5	0 0588	74 8
6	11	1	0	10	17	0 1176	—

angina pectoris before treatment was started in this and Waaler s survey (22)

Infarctions after discontinuing medication

Table IX shows that 12 infarcts occurred after discontinuing therapy They occurred at the following times one after 14 days 2 after 1 2—1 year 6 after 1—2 years and 3 after 2 years or more Most of the patients who suffered from reinfarction after treatment was stopped were patients in the poor risk group

Complications

One of the important objections to anticoagulant therapy is the risk involved, particularly when treatment is not conducted by experts (8 9, 10) A detailed account is therefore given

a) *Hemorrhages* Table XIII shows the number and frequency of bleeding episodes When Quick s method was used the frequency was twice as high as later

The 16 episodes requiring hospital treatment were 5 cases of epistaxis, 6

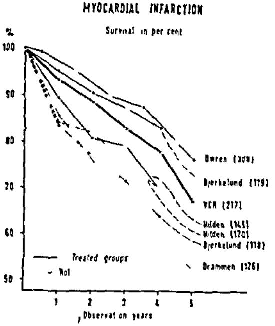
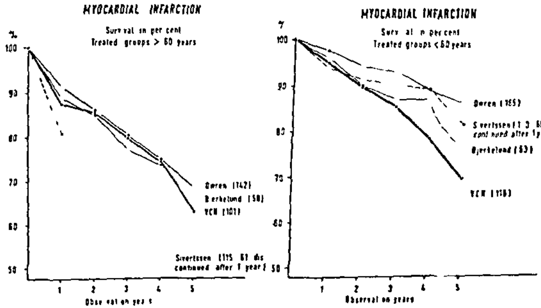


Fig 1 Survival rates in present material (VCH) and 4 other Scandinavian studies See table XV for details

cases of hematuria, 3 cases of gastro-intestinal bleeding and 2 cases of intracranial bleeding, both fatal In the majority of these patients, the laboratory values were below the therapeutic range A local cause of bleeding was found in 4 of the patients, 2 had cystic kidneys and 2 peptic ulcer In one of the patients who died from intracranial hemorrhage, the TT value on admission and also 3 weeks earlier was 19 % Since the patient had hypertension, it is doubtful whether there is any connection between the treatment and the hemorrhages The other patient, however, demonstrates an important but rare bleeding complication *a diffuse subdural hematoma*

The patient, a 64-year old male who started anticoagulant therapy because of myocardial infarction, developed progressive mental disturbances in the absence of known trauma or abuse of alcohol A week later, when admitted to hospital the PP value was 4 % Since neurological examination and FLG supported the diagnosis of



Figs 2 a and b Survival rates for patients with myocardial infarction according to age Present material (VCH) and 3 other Norwegian surveys In the study by Sivertssen et al therapy was stopped after one year in uncomplicated cases

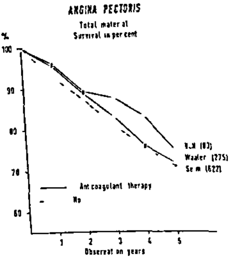


Fig 3 Survival rates for the angina pectoris group (VCH) for Waaler s group (treated) and Seim s group (not treted)

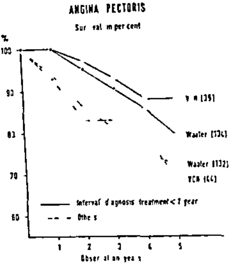


Fig 4 Survival rate for the angina pectoris group in relation to the duration of angina pectoris before treatment was started and cor responding curves from Waaler s study

a deep-seated process probably a neoplasm surgery was not undertaken The patient died within a few days in coma and at autopsy a diffuse fluid subdural hematoma was found

b) *Allergy and other somatic side effects* Allergy to the anticoagulant drug (phe nylindanedione) was demonstrated in 3 patients only The occurrence of diar rhoea has not necessitated discontinuing therapy or been any problem A few men have asked whether phenylindane dione could be responsible for their im potence, but this seems unlikely

c) *Psychoneurotic symptoms* In addition to anxiety concerning their disease and future most of the patients were preoc cupied by and worried over even small fluctuations in the PP(TT) values in spite of repeated assurances that those were harmless and had no connection with their disease A drawback with a standardized well known therapy at an

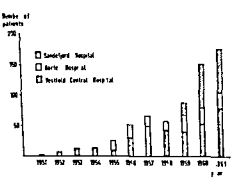


Fig 5 Number of coronary patients starting long term anticoagulant treatment yearly in a population of 120 000 inhabitants in Vestfold county

out patient clinic is that it is very easy for the patient to detect other patients suffering from the same disease When the district is small, as in this case, their anonymity is difficult to protect As all the announcements of deaths appear in the local paper it is inevitable that this situation involving a disease with a high

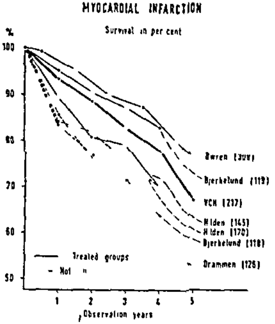
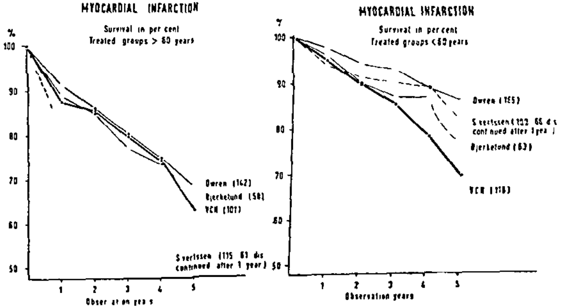


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Figs 2 a and b Survival rates for patients with myocardial infarction according to age Present material (VCH) and 3 other Norwegian surveys In the study by Sivertssen et al therapy was stopped after one year in uncomplicated cases

higher than reported from Sweden by Biorck (1) from 1955—59 15—20 per 10 000)

Table XIV shows the part of the weekly work at the Medical Out patient department occupied by anticoagulant therapy of coronary patients Over 1/3 of consultations consist of such controls and over 40 % of the income or one can say the Health Service's expenses for internal medical specialist control at the out patients clinic, comes from these follow ups During 1951—1956 the total expenses for treatment of one patient (consultations, laboratory tests, drugs and travelling expenses) were from 400 —500 kroner per year By increasing the interval between visits to 8—10 weeks the expenses per patient per year were reduced to about 250 — kr, i e in all about 50,000 — kr for approximately 200 patients who received such therapy in Tonsberg in 1962

Discussion and conclusions

This survey shows that it has been possible to carry out well controlled permanent anticoagulant therapy at an out patient clinic with a changing staff of doctors serving a Norwegian urban and rural population and that the control of these patients is the most common type of consultation at the clinic today

In spite of increasing the interval between controls to 8—10 weeks in stable patients and maintaining lower PP(TT) values than recorded from several studies in this country (2 7 13), there have been few hemorrhages (table XV) A possible explanation of this good result may be that the direct contact between patient and doctor has prevented misunderstandings and has facilitated an early reduction of dosage when there has been heart failure, infections, use of antibiotics, etc The reliable laboratory control is also considered to be important It is unlikely that significant bleedings have been overlooked, because notes were written at all control visits

It is very difficult to draw any conclusions about the *effect of treatment* in a survey such as this, or to compare the results with similar surveys Differences in selection of patients, in age and sex distribution, in the frequency of previous coronary artery disease etc (table XV), demand *great care in making comparison* But, if the material is divided up partly according to age and partly according to the duration of the illness and allowances are made for the small numbers remaining after some years, prognosis for the infarct patients has been approximately the same as for Bjerkelund s treated patients (2) and better than for the patients in his control group and for the untreated patients from Tønsberg s neighbouring town, Drammen in the years 1936—51 (14) On the other hand the prognosis for infarct patients under 60 has been *worse than in Owren s survey* from 1948 —15 (16) The difference is probably due to differences in selection of patients

The survival in the angina pectoris group has been very like that in Waaler's survey (22) and not very different from that of the untreated patients from Drammen published by Seim (19) The

TABLE XIII Bleeding complications (all patients during 900 treatment years). The minor bleedings were minor epistaxis and bleeding from hemorrhoids or gingiva, ecchymosis and slight hematuria or hematospermia

	Treatment years/episode
No. of pat. with hemorrhagic episodes	53
No. of episodes	60 15
Minor hemorrhages	53
Major hemorrhages	19 42
Hemorrhages necessitating hospitalization	16 56
Hemorrhages necessitating cessation of therapy [1]	10
Fatal hemorrhages	2

[1] Including 2 patients with cystic kidneys

TABLE XIV Distribution of consultations and income during a normal week at the out-patient clinic

		% distribution of	
	No. of consultations	Consultations	Income
Anticoagulant controls	26	37	42
Diabetic controls	15	21	19
Other consultations	30	42	39
Total	71	100	100

mortality rate, causes considerable psychological trauma. This was evident from the patients' remarks, but difficult to express in numbers. The use of sedatives might give an indication of the psychoneurotic symptoms, and it was found that at least 25 per cent of the patients used such drugs periodically.

Some socio-medical aspects concerning long-term anticoagulant treatment

Longterm anticoagulant therapy in coronary disease in this county has been more and more widely used during the period of this survey. Fig. 5 shows the number of patients who started such therapy at this out-patient clinic. However, the population served by the clinic has diminished during the period of the survey, new out-patient clinics having been started in Horten in 1955 and in Sandefjord in 1959. To get an impression of the use of this therapy in the population originally served by the out-patient clinic in Tønsberg alone, the number of patients starting therapy at these new clinics should be included. This has been done in fig. 5. In this population of 120,000 inhabitants (which has changed little during the survey) long-term anticoagulant therapy has not been used outside the 3 centers. (The southern part of the county has been served by Larvik hospital.) The number of patients *starting* therapy yearly has increased from 2 per 10,000 in 1955 to 15 per 10 000 in 1961, when 40—45 per 10 000 were *receiving* such therapy.

Taking into consideration the primary mortality of acute infarction and the number of patients with infarction not given anticoagulant therapy, it is possible to calculate roughly the *risk of infarction* in the population as being 20—25 per 10 000 in 1960 (a little

parently given just as intense therapy, but his patients were more selected More investigations in this field seem desirable

In this survey, as in those of Bjerkelund (3, 4) and Nordøy (13), there was no difference in the PP (TT) % levels of surviving patients compared with those who died, nor have the values in association with recurrences been higher than in the total treatment period

Discontinuing therapy in a group of infarct patients did not result in a higher frequency of recurrence than during the period on treatment The group of patients discontinuing was similar to the total group regarding previous infarctions but the patients were older Similar comparison for the angina pectoris group is not possible, since the group discontinuing is very small and the frequency of previous infarctions proved to be much higher than in the total group There were no accumulations of infarcts after discontinuing therapy This is in agreement with several recent reports (4 10, 20) but in contradiction to early reports on anticoagulant therapy (16 22)

The only important somatic complications in this survey were the hemorrhages of which the case of subdural hematoma is of special interest Spontaneous subdural hematomas in patients on anticoagulant therapy have been reported by Strang and Tovi (21), who stress the importance of taking this diagnosis into consideration in patients on anticoagulant therapy who develop cerebral symtoms

In recent years several workers have doubted the value of long term anticoagulant therapy, kept up for years, in patients with uncomplicated myocardial infarction (3, 10, 20), which has been the *main indication* for such therapy at this clinic The results from this survey compared with a similar study (20) from a neighboring town, where patients with uncomplicated myocardial infarction stopped treatment after 1 year, do not seem to favor the use of anticoagulant therapy in such patients for more than a year, at least not in patients under 60 years

Based mainly on the studies of Borchgrevink (5, 6) it is recommended today that the highest priority regarding anticoagulant therapy should be given to patients with angina pectoris of short duration and the treatment should be very intense, aiming at TT values between 10 % and 15 % (7, 17) To carry out such a program in a population will create great diagnostic, practical and economic problems At an out patient clinic like the one in this study, it will also be very difficult because of shortage of doctors, at least if there is to be no restriction of the use of anticoagulant therapy in uncomplicated cases of myocardial infarction To discontinue the therapy, particularly in patients who have been treated for years, requires great tact and is often difficult, since the purpose of the therapy is well known to the patients and their confidence in its effect is often exaggerated In this respect, it is important to notice what Owren emphasizes in a survey of problems of thrombosis (18) that it is unfortunate that the term hypercoagulability is used to mean increased tendency to thrombosis since plasma co-

TABLE XV Survey of the most important data from some of the material and the results in the Scandinavian investigations in fig 3 Several differences in the patient groups are evident

	Infarct patients				Angina pectoris patients			
	Anticoagulant therapy			No antico ag therapy	Anticoagulant therapy			No antico-ag therapy
	Bjerkelund (1950—53)	Hilden et al (1955—58)	Lund Johansen (1951—61)	Olsen et al (1936—51)	Waaler (1948—55)	Borchgrevink (1957—60)	Lund Johansen (1951—61)	Seim (1933—52)
No of pat at start	126	145	217	126	275	103	83	603 (690)
Treatment years from start (total and mean)	*418* (3 5)	*362 5* (2 5)	*611 5* (2 8)	—	*670* (2 4)	*142* (1 4)	*289 5* (3 5)	—
No in whom treatment stopped	7	21	34	—	10	2	13	—
Alive after 3 years	*87*	*56*	*100*	—	*84*	—	*43*	—
Alive after 4 years	43	28	70	—	42	—	34	—
Alive after 5 years	10	12	43	—	19	—	22	—
No of pat								
< 50 years (%)	14	15	17	12	16	18	20	12
< 60 years (%)	51	48	53	39	54	68	58	46
< 70 years (%)	91	85	92	77	91	100	94	82
Ratio men women	3 2 1	4 2 1	9 3 1	1 8 1	6 4 1	4 1	4 9 1	2 1
Previous infarct (%)	13	17	10	0	24	28	17	10
Verified infarctions per treatment year	0 045	0 091	0 059	—	0 034	0 014	0 041	—
Cardiac deaths per treatment year	0 041	0 086	0 079	—	0 051	0 007	0 052	—
Bleeding frequency (number of treatment years)	1 8	1 3 6	[1]1 15	—	1 8	1 14	—	—
Hospitalization because of bleedings (number of treatment years)	*1 21*	*1 13*	*[1]1 56*	—	*1 45*	*1 17*	—	—
Deaths because of cerebral haemorrhage	4	1	[1]2	—	3	0	—	—

[1] Infarct+angina patients 900 treatment years

frequency of recurring infarction was 0 041 per year against 0 034 in Waaler's survey, but three times as high as in Borchgrevink's intensively treated group (5) i e 0 014, in spite of the fact that the patients in our survey were ap-

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The Work and Rehabilitation of Patients with Coronary Heart Disease from an Urban and Rural Population of Norway

By

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This report gives a survey of the work and rehabilitation of the patients described in the previous report

General information

Vestfold county is characterized by having nearly one-quarter of its adult male population directly or indirectly employed in the merchant navy or in the antarctic whaling fleet Industry and transport facilities are well developed There have been no rehabilitation centers in the district but there are two occupational advisers and two sheltered industries for disabled workers Several factories have tried to reserve light jobs as watchmen storekeepers etc for their own disabled employees

The patient's work

Table I gives a survey of the patients work and table II shows the patients type of work graded according to physical effort From Tonsberg and surroundings data regarding the distribution of occupation in the population were available (11) and table III gives a survey of these data and the work of the patients from this population There are relatively more patients from commerce merchant navy and administration than from agriculture forestry building and construction Among the patients who were seamen there were relatively many officers *even when* age is taken into account

Although the numbers are small they support Otterland's observation concerning the mortality of coronary heart disease among different groups of seamen (15)

A Rehabilitation of the male patients

The following survey includes males under 70 years of age who are not pensioners Immediately before *the start of* therapy, 163 *of the male* infarct patients *and 62* of the angina pectoris patients were working and had not planned to retire within the following *6 months*

Patients who resumed work

In the infarct group 75 % (122 patients) resumed work and in the angina pectoris *group 81 %* (*50* patients) More than *91 %* resumed their previous jobs some however with minor modifications

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agulation is only one of the processes leading to thrombus formation and hardly the most important.

Summary

This report gives a survey of the experiences from the first 12 years of anticoagulant therapy of coronary patients at an out-patient clinic with a changing staff of doctors serving a Norwegian urban and rural population. In spite of rather intense therapy (mean PP(TT) value of 18.2 %) and increasing the interval between controls to 8—10 weeks, there have been few bleeding episodes. One of these, a fatal case of subdural hematoma, was probably caused by the therapy. A survey of the prognosis is given. *There was no difference in the mean PP(TT) % between the surviving patients and those who died of infarction, nor in the distribution of these values. Nor did the distribution of values associated with recurrent infarctions differ from the general distribution. In a group of patients discontinuing therapy for various reasons, there was no accumulation of recurrences shortly afterwards, and cessation of therapy did not seem to increase the frequency of recurrences in patients who had started therapy because of recent infarction.

The number of patients starting long-term anticoagulant therapy each year has increased greatly during the period of the study, the main indication being recent myocardial infarction. Problems concerning indications for and cessation of long-term anticoagulant therapy are discussed.

Acknowledgement

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References

References are found at the end of the next article (The work and rehabilition of patients with coronary heart disease from an urban and rural population of Norway), p. 62.

the angina pectoris group were still working when the material was reviewed Of the rest a few had reached retiring age but the majority had stopped work on account of coronary artery disease or had died Of 82 infarct patients who were working, 19 had difficulties on account of chest pain or shortness of breath

All the surviving patients who were under 60 years but had retired had done so because of their coronary artery disease All had been manual workers or seamen It is possible that, with careful placing more of these could have remained employed

Patients who did not return to work

Forty one infarct patients and 12 angina pectoris patients did not return to work Almost half of them were over 60 years old Over 90 % had been doing manual work, though few of them heavy More than 1/3 were seamen Of these 53 patients 32 were alive when the material was reviewed Ten of these 32 were seamen many with slight symptoms or symptom free, and some of these should have been able to work

Of the 22 who had had work on land, there were only 3 under 60 years whose symptoms and physical signs were so slight that they should have been able to work Two of the three lacked interest in resuming work

B Women's ability to work

There were 32 women under 70 years of whom 25 did housework and 7 worked outside the home Among the housewives 3 were completely unable to work after starting therapy, the other 22 returned to housework to a greater or lesser extent, though many could not do shopping, floor washing etc

Of the 7 who worked outside the home, two stopped working after starting therapy, 61 and 66 years old Both had light sedentary jobs The other 5 remained in the same job (4 light work, one non manual work) and except for one who had reached retiring age, all were still working by Jan 1, 1963

Discussion and conclusions

In a somewhat similar survey from another Norwegian county (Rogaland) in 1956—58 Bro trup Knutsen (12) found that of 101 male infarct patients under 67 years of age who were discharged on permanent anticoagulant therapy, 80 % resumed work, and he mentions that in similar surveys the results has been 75 %—80 % The author refers to a large percentage of the patients being self-employed which may account for the favorable result in his survey The poorer results in Vestfold were caused by the large group of seamen Excluding seamen, 79 % of the infarct patients resumed work

Since we became aware of the seaman s special problems in finding work ashore, many have discontinued anti coagulant therapy after a few months and later gone back to work at sea Three patients went to sea on anticoagulant therapy but one of these had hematemesis while at sea, so this is obviously risky

Brostrup Knutsen stresses that many coronary patients are absent from work too long He found that only 66 % of

TABLE I The patients' work

	Men		Women	
	Infarction	Angina	Infarction	Angina
Industry	60	20	—	—
Agriculture/forestry	18	7	—	—
Building and construction	11	2	—	—
Whaling/fishing/sea transport	29	13	—	—
Other transport	17	10	—	—
Administration/armed forces/teaching	21	6	3	—
Health service	1	—	—	1
Hotel/restaurant	1	—	—	—
Business	33	10	1	—
Other	5	1	17	11
Total	196	69	21	14

TABLE II Type of work in relation to grade of physical effort (males)

	Infarct group	Angina group
Heavy work	40	15
Light work	109	39
Non manual work	47	15
Total	196	69

Graded according to the physical effort required, the percentage of the patients resuming work in the infarct group were 72, 71 and 78 respectively for patients with heavy, light and non manual work. The corresponding numbers in the angina pectoris group were 100 (!) 70 and 92.

It was considerably more difficult for seamen to return to work than for other groups. Only 58 % of the seamen with infarction, and 62 % of those with angina pectoris resumed work, compared with 79 % and 86 % respectively of those with other work.

TABLE III Type of work for whole employed population and for the patients in Tonsberg and surroundings

	No coronary pts	No employed	No pts/1000 employed/year
Industry	65	6 500	1.0
Agriculture/forestry	18	3,100	0.6
Building and construction	8	1 700	0.5
Whaling/fishing/transport	50	5 800	0.9
(sea transport alone)	32	2 200	1.5
Administration/armed forces/teaching/professionals	23	2 000	1.2
Health service	2	400	0.5
Business	34	2,000	1.7
Other	22	18 600	0.1

Primary absence from work

About 85 % of the patients who returned to work had been absent for less than 6 months. Fourteen patients had been absent over one year and of these 6 were sailors, 5 had heavy work, 2 light work and 1 non-manual work. The heavy workers did not have striking complications but fear that hard muscular effort would bring about infarction was the main cause for the long absence from work. Of these 14, 5 died during the observation time, 3 reached retiring age and 6 are working without difficulty.

Ability to continue working

Of the patients who returned to work 67 % in the infarct group and 52 % in

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Acute Renal Insufficiency in Intoxication with Mercury Compounds

I Aetiology, Clinical Picture, Renal Function

By

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Since December 1955 up to the end of 1962 at the Second Medical Clinic of the Charles University we treated 48 patients intoxicated with mercury compounds, 40 of whom developed anuria As far as we know it is the largest series of anuric patients described in the literature in general (3 4 6, 7, 8 9) and in a department equipped with an artificial kidney in particular (1, 5) In a number of our patients functional examinations of the kidneys were made during the diuretic stage

We assume therefore that a more detailed study of these patients can reveal not only the typical picture of acute renal failure but can provide also more accurate information on the course and treatment of acute intoxication with mercury compounds

Aetiology

The total of 48 patients included 32 (67 %) women and 16 (33 %) men aged 16 to 60 years The average age was 26 2 years Six teen patients (33 %) were engaged in the health services The cause of intoxication in 40 (83 %) was suicide and in eight cases (17 %) accidental In 46 patients the poison was taken by mouth One female patient used corrosive sublimate solution simultaneously for abortion In one patient the intoxication developed after irrigation of the urinary bladder with oxycyanate and in one patient after an intramuscular injection of a mercury diuretic Corrosive sublimate was used thirty times oxycyanate fourteen times mercuric oxide three times and salyrgan once

Submitted for publication June 15 1964

Clinical picture

Period of shock and toxic symptoms Symptoms of shock were recorded seven times A metal flavour was reported by only three of our patients Inflammation of the oral cavity was observed in twenty three patients Pain in the epigastrium and subsequent vomiting several minutes to half an hour after ingestion of the poison was reported by 44 patients haematemesis by 21 Diarrhoea was reported by more than two thirds (33)

those who resumed work had done so during the first 6 months, against 85 % in our study In both series the patients *with heavy manual work* were absent for a long time, not because of severe symptoms, but because they feared that hard muscular effort would cause recurrence The survey has shown the need for better rehabilitation facilities, particularly for seamen The problem is greatest for captains and mates having highly qualified work with a high salary at sea and great difficulties in finding suitable work ashore

Summary

A brief survey of the type of work and the ability to resume work is given for a group of coronary patients from a Norwegian rural and urban population Attention is drawn to the special problem coronary disease creates for seamen and whale fishers

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blood in the faeces by 21 patients We did not show a relationship between the intensity of symptoms and the amount of ingested poison

Period of acute renal insufficiency (fig 1) Anuria developed always within 24 hours after intoxication The length of anuria (4—29 days, average 9 5 days) was unrelated to the amount of ingested poison (0 1—8 0 g) Initial polyuria was not observed in any of the patients The non protein nitrogen reached an average value of 224 mg % with a maximum of 372 mg % Hyperazotaemia persisted on average for 31 days with a maximum 79 days We were unable to provide evidence of a relationship between the length of anuria and azotaemia ($r = 0.775$ $p > 0.05$)

Hyperkalaemia (> 5 5 mEq/l) was found in 17 patients Hyperkalaemic changes on the electrocardiogram were mild (spiky T wave) We found a close relationship between hyperkalaemia and hyperkalaemic changes on the electrocardiogram ($chi^2 = 8.05$, $p < 0.01$)

Plasma chlorides and sodium were reduced (average 94 4 mEq/l $s = 9.36$ mEq/l and 124 5 mEq/l, $s = 8.38$)

As regards blood proteins examined by paper electrophoresis, a marked drop of albumins was noted (minimal average value 36 3 %) and the $alpha_1$ $alpha_2$ beta and gamma globulins increased (maximum average values 10 4 13 9 15 6 30 1 %) The lowest albumin values were found about the sixteenth day of the disease and the values returned to normal about the 40th day of disease We were unable to provide evidence of a relationship between albuminaemia and the level of the non protein nitrogen We found a relationship between the lowest albumin values and the period of protein deprivation ($r = 0.913$, $p < 0.01$)

Clinical signs of uraemia were mild The cardiovascular system was not impaired The sedimentation rate of red blood cells usually increased to values of about 100 after one hour with maximum values on the 18th day of the disease Raised values persisted for another two or three weeks

Anaemia was normochromic or slightly hypochromic On an average after 28 days the blood haemoglobin values dropped to 8 7 g/100 ml blood The return to normal values took 1—2 months The drop of haemoglobin was only roughly related to the non protein nitrogen blood level ($r = -0.635$, $p = 0.05$) We found no relationship between the lowest haemoglobin values and the persistence of raised azotaemia ($r = -0.227$, $p > 0.1$)

The number of leukocytes in the blood in anuric patients was always raised There exists however no relationship between the number of leukocytes and the duration of anuria ($r = 0.332$, $p > 0.1$)

Proteinuria (never exceeding 1 g/24 hours) disappeared about the 23rd day of the disease

Renal function

We made the concentration test, investigated the creatinine clearance, inulin clearance, the clearance of para aminohippuric acid (PAH), Tm_{PAH} and the extraction of para aminohippuric acid (E_{PAH}) by commonly used

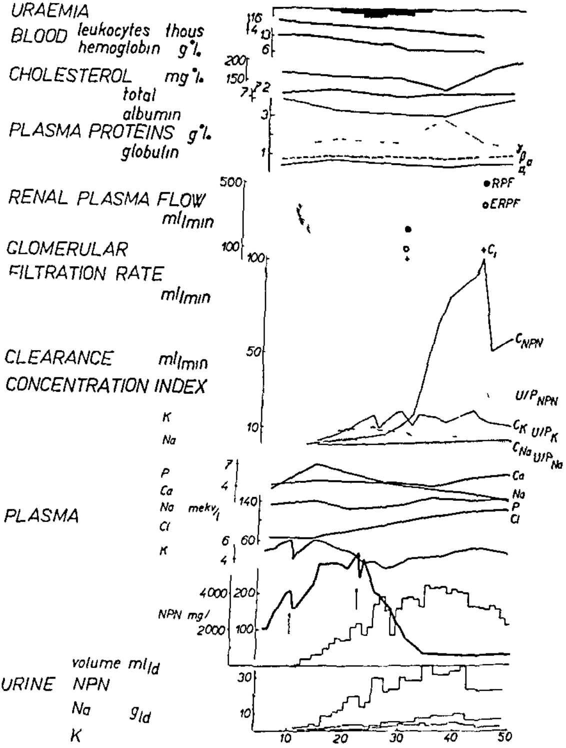


Fig 1 Course of disease Arrows in non protein nitrogen curve indicate dialysis On *x* axis are the days of the disease The figure illustrates the course of the main clinical and laboratory indicators of the disease Clinical signs of uraemia were only slight The patient suffered from anorexia vomiting and mild apathy

urinary output greater than 1 ml/min and the data are thus not influenced by technical errors due to small amounts of urine

Large inulin molecules probably do not penetrate through the damaged wall and inulin clearance can be considered as an indicator of glomerular filtration During the first days of diuresis the proximal tubule is passively permeable for para aminohippuric acid in both directions The ratio of inulin clearance and clearance of para aminohippuric acid on the 7th and 9th day of diuresis is greater than 1, Tm_{PAH} on the seventh day is negative The excretion of para aminohippuric acid through the kidneys is so slight that the difference in concentrations in arterial blood and blood from the renal vein is within the range of the error of chemical estimation method and F_{PAH} (4th and 7th day) thus cannot be used for assessing the total renal blood flow

At the onset of the diuretic stage all examined values were markedly reduced

Inulin and creatinine clearance rise rapidly and during the second month they reach normal levels Somewhat later the clearance of para aminohippuric acid as well as the total renal blood flow reach normal levels The maximum secretion of para aminohippuric acid (Tm_{PAH}) returns to normal levels only during the fifth months of the disease (fig 3)

Summary

During 1955 to 1962 a total of 48 patients after acute intoxication with mercury compounds were treated Forty patients developed anuria, persisting for 4 to 29 days

Aetiology, clinical picture and renal function are presented

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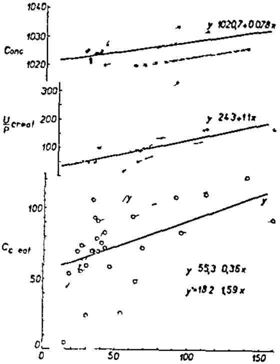


Fig 2 Adjustment of creatinine clearance and concentration capacity during diuretic stage of acute renal insufficiency Conc maximum concentration of urine attained during concentration experiment U/P creat creatine concentration index C_{creat} creatinine clearance in ml/min On x axis are days of the disease Heavy lines are regression curves Y in the creatinine clearance is the regression line which defines the relationship between the creatinine clearance value and the day of disease in all patients and all examinations Y is the regression line for the examination made at the beginning of the late diuretic stage i e on the 18th to 46th day of the disease The slope of the two curves indicates that the adjustment takes place approximately at the same rate as the biological curve

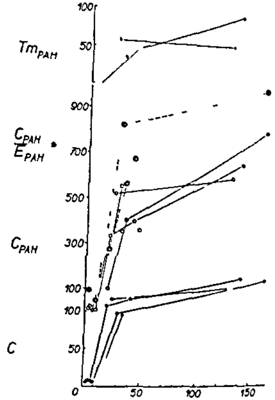


Fig 3 Adjustment of inulin clearance clearance of para aminohippuric acid extraction of para aminohippuric acid and Tm_{PAH} This figure illustrates the marked decrease of all functions at the onset of the diuretic stage and the rapid adjustment Tm_{PAH} is slowest in reaching normal levels

methods (2) Specimens from the renal vein for assessing the E_{PAH} were obtained by intubation via the femoral vein

The clearance of endogenous creatinine increases proportionally with time from the onset of the disease ($r = 0.835$, $p < 0.01$), as well as after the end of anuria ($r = 0.832$, $p < 0.01$) We did not find a statistical relationship between the duration of anuria and the rate of renewal of renal function ($r = 0.418$, $p > 0.05$) The clearance of endogenous creatinine did not return to normal before the 35th day of the disease

The concentration capacity of the kidneys improves parallel with time ($r = 0.86$, $p < 0.01$), the rate of return to normal of the concentration capacity does not depend on the length of anuria ($r = 0.333$, $p > 0.05$) The concentration capacity did not reach a normal level before the 48th day of the disease, as a rule between the 60th and 90th day of disease (fig 2)

The examination of inulin clearance clearance of para aminohippuric acid, Tm_{PAH} and E_{PAH} was made during a

urinary output greater than 1 ml/min and the data are thus not influenced by technical errors due to small amounts of urine

Large inulin molecules probably do not penetrate through the damaged wall and inulin clearance can be considered as an indicator of glomerular filtration During the first days of diuresis the proximal tubule is passively permeable for para aminohippuric acid in both directions The ratio of inulin clearance and clearance of para aminohippuric acid on the 7th and 9th day of diuresis is greater than 1, Tm_{PAH} on the seventh day is negative The excretion of para aminohippuric acid through the kidneys is so slight that the difference in concentrations in arterial blood and blood from the renal vein is within the range of the error of chemical estimation method and E_{PAH} (4th and 7th day) thus cannot be used for assessing the total renal blood flow

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Summary

During 1955 to 1962 a total of 48 patients after acute intoxication with mercury compounds were treated Forty patients developed anuria, persisting for 4 to 29 days

Aetiology, clinical picture and renal function are presented

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Acute Renal Insufficiency in Intoxications with Mercury Compounds

II Treatment and Prognosis

By

ALBERT VALEK

Treatment of acute renal insufficiency in intoxications with mercury compounds is one of the most difficult and when successful one of the most pleasant tasks of the physician Young healthy individuals who suddenly fall acutely ill can if the correct procedure is applied, recover completely and become again useful members of human society This is why so much attention was paid to this problem (4, 7, 10 12, 14 16 17, 18, 19)

But only when an effective antidote — dimercaptopropanol (BAL) (8 9 22) was discovered and when new physiological findings were applied in practice (5 6 15) and when it proved possible to design an artificial kidney which can be used in man (1 13) were conditions created for reducing the mortality in very severe intoxications

At the Second Medical Clinic of the Charles University in Prague we treated 48 patients between 1955 and 1962 with acute renal insufficiency caused by intoxication with mercury compounds In the present paper we are presenting a report on the methods and results of treatment

Submitted for publication June 15 1964

Material and methods

Our patients can be divided into four groups

a) Those who did not develop anuria (less than 100 ml/24 hours) or oliguria (less than 300 ml/24 hours) and who recovered without special treatment (7 patients),
b) One patient died from shock and was treated according to principles valid for the treatment of shock of different aetiology
c) Patients with anuria or oliguria lasting 4—12 days treated only conservatively (16 patients)
d) Patients with anuria or oliguria lasting 6—29 days where in addition to conservative treatment haemodialysis by means of an artificial kidney was also used (24 patients)

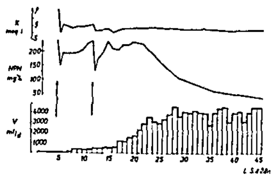


Fig 1 Effect of haemodialysis on non protein nitrogen and potassium level in the course of the disease
K serum potassium, NPN non protein nitrogen in blood, V ml/d amount of urine excreted per day in ml On *x* axis days of the disease are given The arrow indicate dialysis During the first dialysis the blood potassium level declined from values threatening *life to normal* ones and later it did not change any further Both dialyses, by reducing the non protein nitrogen level in blood and by adjusting the milieu intérieur, helped the patient to overcome *the period of anuria and oliguria*

All intoxicated patients vomited within 30 min after ingesting the poison, the stomach was irrigated in 24, BAL was administered within 24 hours to 36 patients

During anuria or oliguria the patients were given a highcalorie diet lacking protein, potassium and salt Most patients, however, did not eat at all or ate very little Therefore we administered daily 500 ml 20 % glucose with 30 u insulin, 30 ml 10 % calcium gluconate and vitamin C by drop infusion for 8—10 hours, vitamin B_1 100 mg i m, methylandrostendiol 25 mg/day The increased fluid requirements were covered by drinking, in those who vomited by the parenteral route Antibiotics and blood transfusions were prescribed as needed The patients were encouraged to sit up and to walk

During the diuretic period the condition of the patients improved rapidly, they ceased to vomit and their appetite returned and we were thus able to discontinue infusions and were able to cover the loss of fluids by the oral route As soon as the diuresis reached one litre, we gave the patients a salt containing diet with meat and in case of hypokalaemia we added *8—10 g potassium chloride* by mouth

The dialyses were carried out by means of an artificial kidney of Alwall s type Dialyses were indicated in hyperkalaemia exceeding 6 5 mEq/l Signs of hyperkalaemia on the ECG supported our decision Dialyses were also used in clinically manifest uraemia and hyperazotaemia above 200 mg % when there was no hope of an early rapid drop of the non protein nitrogen, even when the the patient lacked signs of uraemia or hyperkalaemia

In most patients the blood was conducted into the apparatus from the radial artery and returned into the cubital artery During the past year we used the veno-venous method with percutaneous puncture of the femoral vein and two catheters which were inserted into the v cava caudalis (20) (fig 1)

The clinical condition of the patients and progress were followed by the usual physical and laboratory examinations

The *degree of catabolism* was assessed daily by estimating the non protein nitrogen and potassium blood level, in hyperkalaemia we made electrocardiographic tracings every *day The water balance was checked* by weighing In all patients we examined the plasma proteins daily Once a week we made X rays of the lungs in order to detect *in time oedema of the lungs or* broncho pneumonia physically not manifest

Results

1 *Irrigation of stomach and dimercaptopropanol (BAL)* We did not find a statistically significant difference in the length of anuria between patients treated and not treated with BAL in those patients subjected to irrigation of the stomach ($t = 0.616$, $p > 0.5$) On the other hand in patients whose stomach had not been irrigated BAL had a significant influence on the duration of anuria ($t = 4.52$, $p < 0.01$)

2 *Conservative treatment of anuria* before admission to the clinic, i e in anuric patients not treated by the ad ministration of hypertonic glucose the daily increment of non protein nitrogen in plasma was 28 mg %, in patients admitted to the clinic and treated with 20 % glucose it was only 17 5 mg % This difference is statistically significant (t = 3 25, p < 0 01) Hypertonic glu cose also influenced the hyperkalaemia (chi² = 11 85, p < 0 01)

3 *Haemodialysis using the artificial kid ney* the clinical picture and course of renal failure was more severe in this group of patients than those not sub jected to dialysis Hyperazotaemia and hyperkalaemia reached higher values and the sedimentation rate of red blood cells was markedly higher in the dialysed patients

The effect of haemodialysis on the level of non protein nitrogen and the potassium blood level in the course of the disease is illustrated by fig 1 The course of the dialysis proper is shown in *fig 2*

As we adjusted the period of dialysis and the size of the dialysation membrane as needed we calculated the drop of non protein nitrogen and potassium per hour of dialysis and ten metres of dialysation tube (i e cca 6 500 cm² dialysation area) The plasma concentra tion of non protein nitrogen declined on an average by 11 5 mg/10 m/1 hour on an average 3 7 g/10 m/1 hour of non protein nitrogen was removed by dialy sis In hyperkalaemic patients the plasma potassium declined by 0 26 mEq/l/10 m/ 1 hour and 16 5 mEq/10 m/1 hour potassium was removed

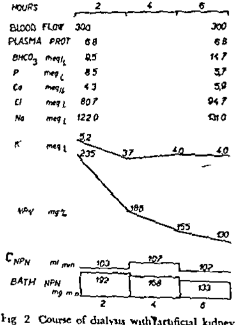


Fig 2 Course of dialysis with artificial kidney in acute renal insufficiency after intoxication with corrosive sublimate

Haemodialysis brought also the re maining plasma ions to normal levels and in most instances somnolence and vomiting receded from the clinical picture if present before

During dialysis the systolic pressure dropped by 14 mm Hg (t = 2 24, p < 0 05) and the mean aortic pressure by 10 mm Hg (t = 2 4, p < 0 05) The diastolic pressure did not change (t = 1 55, p > 0 1) The pulse rate also did not change markedly (t = 1 0, p > 0 2)

Dialysis was complicated only in one instance by a marked drop of blood pressure an increased pulse rate and a short period of unconsciousness After discontinuation of the dialysis and blood transfusion the condition improved with in several minutes We observed how ever repeatedly thrombophlebitis at

TABLE I A Influence of length of anuria on mortality

No of days	No of patients	Died	%
<6	5	0	0
6–10	28	5	18
11–15	7	3	43
>16	2	1	50

the site where cannulae were left in the veins after dialysis to facilitate parenteral treatment

4 *The influence of antibiotics on complications* Bronchopneumonia was not influenced by broad-spectrum antibiotics, even when administered in high concentrations Thrombophlebitis responded very well and improved within several days

Discussion (table I A and B)

Anuria after intoxication with mercury compounds is always a serious threat to life Before the Second World War the mortality was 55—100 % (4, 7, 21)

Hull and Monte (11) between June 1917 and June 1933 gathered a total of 302 intoxications with corrosive sublimate Of these 15 patients died from shock A marked reduction of the diuresis or anuria lasting for less than 24 hours was found by these authors in 27 patients Of these 15 died Anuria lasting for 24 hours or more was found in 40 patients Of these 37 died (92 5 %) No patient who did not pass urine for more than three days survived and some died during incipient diuresis All these patients died from uraemia Only in five patients from the entire group is another cause of death given once haemorrhage from the gastrointestinal tract, twice gastrointestinal disorders not specified more closely but lacking signs of uraemia, and twice the cause was unknown

Marked hyperazotaemia was always due to oliguria or anuria and was an adverse prognostic sign From those

TABLE I B Influence of hyperazotaemia on mortality Comparison of group described by Hull and Monte (11) of 1917—1933 and ours of 1955—1962

Maximum value of hyperazotaemia (mg %)	Hull Monte: Total no of patients	No of deaths	% of deaths	Our group: Total no of patients	No of deaths	% of deaths
< 50	21	1	5	7	0	0
51–100	25	4	16			
101–150	10	7	70	15	0	0
151–200	8	6	75			
201–250				11	2	18
251–300	8	8	100	11	6	55
>301	5	5	100	3	1	33

(In the Hull Monte columns, 201–250 and 251–300 are bracketed together; in our group, 51–200 are bracketed together with the 101–150 row values.)

patients where the maximum non protein nitrogen level was above 100 mg % more than 70 % died, among those with values above 200 mg % none survived

As compared with these data, from our 48 patients 10 died, the total mortality is thus 20 8 % One patient died from shock 55 hours after the intoxication, in the remaining 9 patients anuria persisted for more than 6 days The mortality of the anuric patients is thus 21 4 % In the anuric stage, on the 9th day, one patient died, the remainder at the onset of the diuretic stage 14 to 29 days after the intoxication

Seven patients died from bronchopneumonia, one from massive haemorrhage from the gastrointestinal tract and one female patient from septicaemia All patients who survived recovered completely

With protracted anuria and rising hyperazotaemia the prognosis of the disease deteriorated The difference between the highest non protein nitrogen level in the group of patients who died (281 mg %) and patients who recovered (228 mg %) is statistically significant ($t = 2.62$, $p < 0.01$) The number of days when the non protein nitrogen was above 200 mg % is also much higher in the patients who died (average 8 38 days) than in the controls (3 17 days)

Our patients survived thus several times longer periods of anuria and a higher hyperazotaemia than Hull and Monte s patients (11) and none of them died from uraemia The causes of death were complications, mostly bronchopneumonia

The difficult diagnosis of pneumonia and the ineffectuality of antibiotics indicate that it is important to focus attention in the first place on prevention Respiratory exercise, gymnastics and great care of the nursing staff as regards the upper respiratory passages seem to be one of the most feasible approaches (2)

The correct evaluation of the clinical picture and modern therapy which is capable of keeping alive anuric patients for several months (3) create the conditions for man to survive even the most severe renal damage caused by mercury compounds

Summary

1 The author describes the method and results of treatment in 48 patients after intoxication with mercury compounds Forty patients developed anuria lasting for 4 to 29 days Evidence was provided of the importance of early administration of BAL and irrigation of the stomach During conservative treatment of anuria hypertonic glucose reduced markedly the daily increment of non protein nitrogen in blood Dialysis by means of an artificial kidney was used in those patients where the condition could not be brought under control by conservative methods

2 The mortality was 20 8 % One patient died from shock seven from bronchopneumonia one from haemorrhage from the gastrointestinal tract and one from septicaemia The prognosis was the more adverse, the higher and more prolonged the hyperazotaemia All patients who survived recovered

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51–100	25	4	16	15 (51–200)	0 (51–200)	0 (51–200)
101–150	10	7	70			
151–200	8	6	75			
201–250	8 (201–300)	8 (201–300)	100 (201–300)	11	2	18
251–300				11	6	55
>301	5	5	100	3	1	33

Acta Medica Scandinavica Vol 177, fasc 1, 1965

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Acute Renal Insufficiency in Intoxication with Mercury Compounds

III Pathological Findings

By

Josef Stejskal

The pathology of sublimate poisoning and poisoning by other soluble mercurial salts is well known and studies and experiments on this subject are being continued all the time However, the last extensive morphological study on human material was probably Nakata's work from 1922 At present sublimate poisoning has become a rarity to be seen in the dissecting room and only very few pathologists may ever have the opportunity to see and to compare more cases

Since 1955 when the first artificial kidney in Central Europe started to be used on the II medical clinic of the Faculty of General Medicine in Prague 50 cases of poisoning by toxic compounds of mercury have been treated 11 of which died The clinical picture of this series has been published elsewhere (16) In this study we want to find out how the morbid anatomical picture of sublimate poisoning in humans has changed due to modern therapeutic measures in comparison with the classical one

Material

The morphological pictures of 11 cases of mercurial salt poisoning from the years 1957—1963 are compared with 11 post mortems from the years from 1920—1942, taken from the material of Hlava s institute Clinical data pertaining to the older group of our series were mostly inaccessible

Results

The dose of the poison taken by the deceased — as far as it could be established — was usually a high one, between 1—8 g (table I) Other patients, however, having taken just as high a dose of the poison recovered after dialysis

The majority of patients had taken the poison orally Exceptions are

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Sites of pseudomembran and necrot inflamm	Colour and weight of kidneys (g)	Microsc calcific	Remark
Larynx pharynx stomach ileum	Yellowish 400	++	—
Colon	Whitish rose 300	+++	—
Colon	Beige 250	+	—
Colon	Greyish grown 400	—	—
Oesoph stomach larynx trachea colon	Rose 360	—	Attempted drowning
Colon	Brownish rose 320	—	Abortion with sublimate hysterectomy
Oesoph stomach ileum colon	Sharp red 320	+	+ cutting of veins
Stomach colon	Light brown 340	—	—
Pharynx stomach colon	Greyish white ?	+	Combined poisoning
Urinary bladder	Reddish brown ?	—	Uncleared anuria
Colon	Yellow brown 260	—	—
Colon	Greyish blue 250	+++	—
Stomach ileum colon	Light yellow ?	—	—
Stomach jejunum rectum	Rose brown ?	—	About 1—3 days after poisoning
Stomach colon	Greyish rose 540	—	—
Stomach small and large intestine mouth	Yellow grey ?	—	—
Oesoph pharynx colon	Violet ?	+	—
Stomatitis colon	Light grey 330	—	Marked cachexia and dehydration
Vagina colon gingiva tonsils	Brownish pink ?	—	After introduction of $HgCl_2$ tablet into vagina
Mouth oesophag ileum colon	Brownish pink 250	—	—
Mouth pharynx larynx colon	Brownish pink 390	—	—
Mouth stomach colon	Greyish yellow 260	—	—

TABLE I Cases 1—11 are from the years 1957—1963, cases 12—22 from the years 1920—1942

No	Sex	Age	Poison dose (g)	Survival	Cause of death
1	♀	17	1	14 days	Bronchopn
2	♀	29	8	17 days	Bronchopn bilat
3	♂	17	3 5	26 days	Bronchopn bilat, uraemia
4	♂	26	2	15 days	Bronchopn bil, uraemia
5	♀	26	5	9 days	Bronchopn absced
6	♀	25	?	29 days	Bronchopn absced, peritonitis
7	♂	44	2	55 hours	Shock
8	♂	24	8	18 days	Bronchopn bilat
9	♀	48	3	28 days	Uraemia, sec oxalosis, termin. sepsis
10	♂	40	?	20 days	Uraemia, bleeding
11	♂	54	0 5	17 days	Uraemia, bronchopn
12	♂	19	?	?	Bleeding to death from gastric ulcer
13	♀	32	?	?	Uraemia
14	♂	26	?	?	Lung oedema, brain/shock?/
15	♀	29	1—2	9 days	Bronchopn absced
16	♀	23	?	?	Bronchopn confluens
17	♀	20	2	10 days	Uraemia sepsis from empyema
18	♀	49	5	36 days	Uraemia cachexia colitis ulcer
19	♀	46	1	15 days	Uraemia
20	♀	42	0 5	9 days	Uraemia
21	♀	27	?	?	Uraemia
22	♀	27	1	10 days	Uraemia

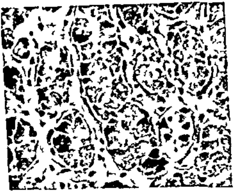


Fig 1 Case 7 Simple necrosis of the epithelium of the proximal tubule 55 hours after poisoning Note the regeneration in the form of flat cells on the membrane Some foci of calcification interstitial oedema Haematoxylin-eosin × 400



Fig 2 Desquamation of the necrotic mass which is facilitated by macrophagic properties of the regenerated cells Interstitial hyperaemia Case 5 9 days after the poisoning Haematoxylin-eosin × 400

In the first days after the poisoning (cases Nos 7 and 14) the well known picture of simple necrosis of the proximal tubule could be observed Underneath the necrotic epithelium a very lively regeneration had started in the shape of flat cells with dark nuclei lying on the basal membrane

During the further phase of the development of changes in the proximal tubule we observed desquamation of the necrotic epithelia and their removal into the more distal parts of the nephron *in the second week* (cases Nos 1, 5 15 17, 20 22 as well as 12 13 16, 21) The regenerated cells presented a very interesting picture growing into the necrotic masses like roots encircling them and desquamating into the lumen together with them Underneath them new flat cells regenerated being often multinuclear and gradually lining the whole circumference of the tubule Mitoses were rare

In the third week (cases Nos 2 4 8 10, 11 19) the proximal tubules were lined with a continuous layer of a low remarkably basophilic epithelium with round, often multiple nuclei, located rather irregularly around the circumference of the tubule The lumen of the tubules was slightly dilated usually empty and residues of the necrotic material were to be found in the cortex only occasionally It was very difficult and often quite impossible to differentiate the proximal part of the tubule from the distal one



Fig 3 Case 8 Continuous layer of basophil epithelium 17 days after the poisoning Note the numerous multinucleated cells and persistent desquamation into the dilated lumen Interstitial oedema Haematoxylin-eosin × 380

A 46-year-old woman belonging to the older group (table I No 19), who by error introduced a sublimate tablet into her vagina, and also a 40 year old man (table I No 10), who died on the 20th day after a lavage of his bladder probably with a mercurial solution (Traces of mercury could be chemically proved in his kidneys)

The length of survival is demonstrated on table I In the older group the patients died mostly on the 9th—10th day, that is during the period of renal failure Death occuring 36 days after the poisoning was an exception (table I, No 18) Our patients on average died at a later time, between the 14th—15th day, that is during the period of convalescence Only one woman, 26 years old (table I, No 5) died on the 9th day from bronchopneumonia and a 44-year-old man (table I, No 7) died on the third day from uncontrollable shock However both patients were cases of combined suicide

The cause of death was uraemia in the majority of cases of the older group, in the recent ones it was usually bronchopneumonia

The clinical and anatomical picture of uraemia manifested itself in 4 patients of the recent group (Nos 3, 8, 9, 11), however, contrarily to the older group, uraemia was not the only cause of death Bronchopenumonia was always very extensive affecting several lobes, mostly in both lungs, abscess formation was frequent Aspiration of vomit was proved with certainty in one case (table I, No 5), it was regarded as probable in further two or three cases (Nos 7, 8, 15)

One patient certainly died from shock after poisoning (table I No 7) another one probably died from this cause (table I No 14) Haemorrhage into the digestive trac killed two patients (table I, Nos 10, and 12) Severe metabolic disturbance with high hyperpotassaemia in terminal sepsis led to the death of a 48 year old woman (table I, No 9) after combined poisoning with a sublimate solution, nitric acid and copper sulphate (a hairdresser s preparation) and manifested itself by secondary uraemic oxalosis (2)

The macroscopic changes in the kidneys corresponded only roughly to the three phases as proposed by Askenazy, quoted by Nakata 1 The red kidney up to 24 hours, 2 The greyish-white kidney from the 2nd—7th day, 3 The red kidney from the 8th day The time limitation of the various phases is not an exact one we were able to find an enlarged kidney of light red colour as late as 55 hours after the poisoning A greyish white kidney with a dark red medulla has been described from the 9th—to the 28th day The colour of the kidney in the third and fourth week has never been described as "red" by the dissector, but as "brownish pink" or "dirty reddish brown" etc The kidneys always displayed marked engorgement and cloudy swelling Considerable congestion of the medulla was usually very marked in most cases The weight of the kidneys varied from 250 to 540 g, being usually around 300—350 g

In the microscopical picture of the kidneys we were able to follow the development of the lesions from the onset of necrosis 55 hours after the poisoning up to the late stages of regeneration after 4—5 weeks The changes did not occur and develop in all cases at the same rate, they probably depend upon the success of the therapy and upon the general state of the patient

were relatively frequently found not only in the interstitium, but also in the lumina of the tubules, mostly in the vicinity of casts A considerable dilatation of capil laries on the border between the cortex and the medulla was a quite constant change in all phases

Calcification of necrotic material was found to a marked extent only three times (cases Nos 1, 2, 12) and occurred occasionally in four cases (Nos 3, 7, 9, 17), that is altogether in less than one third of all cases

The described changes differed quan titatively from one another in the in dividual cases *Differences in the extent of necrosis* of the proximal tubules could be found in addition to differences in cal cification, in the interstitial reaction and the amount of casts In four cases (Nos 1, 12 13, 17) we observed a preserva tion of the juxtamedullary portion of the proximal tubule, especially of the straight portion lying in the striae medullares These parts displayed only hydropic changes

The histological picture in a 49 year-old woman (case No 18) presented more pronounced differences in comparison to more recent cases surviving for a longer time probably due to different treatment She had died 36 days after the poisoning showing marked signs of cachexia and dehydration The tubules in the medulla were filled with many casts and the cortex presented the typical picture of nephrohydrosis with a marked dilatation of Bowman s capsules of the glomeruli and with enormous dilatation of the tubules The epithelium was unusually flat in relation to the time elapsing after the poisoning The interstitium was increased and some leukocytic infiltration could be found in some places



Fig 6 Case 7 Pseudomembranous and necrotizing (arrow) inflammation in the stomach after sublimate poisoning

Pseudomembraneous to ulcerous and necrotizing inflammations could be noted in all cases The colon was affected regularly, in 20 cases of 22 occurring also in such cases where the poisoning had been effected by absorption via the vaginal mucosa The changes were usually most marked in the caecum, *in addition, inflammatory* changes were found in the stomach in ten cases in the proximal part of the digestive tract, in eleven four times in the respiratory tract once in the urinary bladder and once in the vagina Cangrenous changes were found twice, once affecting a small portion of the caecum (No 7) once affecting an oesophageal segment (No 17)

Discussion

The danger of bronchopneumonia as a fatal complication is not only typical of sub limate poisoning, but also of cases of renal failure due to other causes, treated with the artificial kidney In sublimate poisoning of course, frequent and violent vomiting with the danger of aspiration,

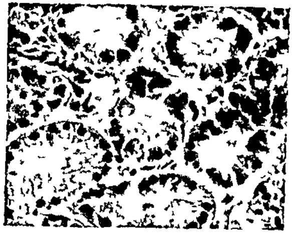


Fig 4 Case 9 The late regeneration of epithelium 28 days after the poisoning Coarser vacuolisation in the plasma Haematoxylin eosin × 400



Fig 5 Case 6 Hydropic alteration of the regenerated epithelium with brush border 29 days after the poisoning Masson s trichrom × 950

In the fourth and fifth week (cases Nos 3, 6, 9, 18) the differentiation of the lining epithelium of the proximal tubule continued in the direction towards its normal appearance The cells were mostly columnar ones, the nuclei round, lighter and located already near the base In some tubules a brush border was noted The plasma was mostly hydropically altered, showing sometimes coarser vacuoles The regenerative process was so far advanced already, that it was not always possible to distinguish the epithelium as a regenerated one Case No 18 was an exception (see later)

Changes of the glomeruli were difficult to differentiate from autolysis Most of the glomerular loops were filled with blood to an increased extent The only change recognizable was an irregular thickening of Bowman's capsule, observed mainly during the first two weeks

Changes in the remaining parts of the nephron were marked especially from the second week after the poisoning, when the distal parts of the nephron were — to a greater or smaller extent — filled with casts consisting of a granular eosinophilic substance and with ghosts from necrotic cells, often surrounded and enclosed in multinuclear cells, which showed regressive changes The surrounding epithelium was greatly flattened, basophilic, with pycnotic nuclei In other places signs of regeneration and frequent mitoses were noted In the further stage considerable individual differences were already becoming apparent In some cases casts were found in the cortex and also occasionally in the medulla (cases Nos 3, 6), in others they were found frequently, especially in the collecting tubules (cases Nos 2, 9, 10) In other cases, however, almost all tubules in the medulla were filled with casts (cases Nos 8, 18)

The interstitium in acute cases especially was usually distinctly dilated and oedematous Cellulization was clearly marked mainly during the second and third week, but not in all cases This was most extensive around the casts and tubules with destroyed epithelium Marked fibroblastic reaction was usually also present in these places (case No 8) Leukocytes

on the length of survival, nor may it be explained by the changes of blood pH and of calcium levels (1)

It is not possible to go into further details on the very interesting problems of the *pathogenesis of sublimate poisoning*, which are very complicated and not solved conclusively as yet Necrosis of the cells of the proximal tubule may develop either by direct toxic effects of the poison upon the cell, or by disturbance of the renal circulation Experimental and morphological proofs are available for both mechanisms In the first case it is likely that the mercury passes through the glomerulus in such a low concentration, that its damage may be detected only by electron microscopical examination (13) and in the proximal tubule the concentration of the poison increases to reach toxic levels (14) The decrease of the blood flow through the kidneys and defects of the filling of peritubular capillaries developing immediately after the poisoning (3, 9) are explained by vascular spasms and indicate the possibility of anoxic damage to the cells of the proximal tubule which are exceedingly sensitive to lack of oxygen A similar mechanism may be supposed to apply also to the colon where mercury may be histochemically demonstrated not only in the necrotic cells of the intestinal mucous membrane but also in the capillaries of the villi of the mucosa and in the submucosal vessels (12, 17) However histological examination of the vascular changes at the sites positive for mercury will reveal paralytic dilatation of the vessels already at the earliest stage of the poisoning The capillaries especially are affected which may also lead to a restriction of the blood flow and to anoxic changes The paralysis of the capillaries causes a decrease in the filtration pressure in the glomerulus, which leads to the early development of anuria

Oliver's tubulorhexis and diffuse ischaemic damage to the distal nephron, supposed to bear some relationship to shock, may be demonstrated in histological sections only with difficulty, using the dilatation and cellular reaction of the interstitium as an indicator, its direct proof being impossible at present We saw such picture in our cases five times (Nos 2, 3, 8, 15, 16), but an unequivocal interpretation of the lesion is difficult because focal pyelonephritis with a progression into the interstitium may frequently develop in anuric kidneys

Pseudomembranous and ulcerative intestinal changes were almost constant findings in our cases even as late as in the third and fourth week after the poisoning Colitis was absent especially in case No 10, where the absorption of the mercury was supposed to have taken place through the urinary bladder, probably coinciding with an intolerance of the patient against mercury Similarly renal damage was observed to occur following small doses of the poison given parenterally without development of colitis (5) Colitis without renal damage, e g after older types of mercurial diuretics (7), is not as easy to explain

A slight accumulation of mercury in Kupffer's cells in the liver may be demonstrated histochemically only during the first days after poisoning (17) The amount of mercury in the liver and in other organs decreases however in a few days to insignificant values as has

plays an important role in the general weakening of the organism

The main morphological differences between the old and the recent group of cases are to be found in the kidneys, being represented by the finding of advanced regeneration in the third up to the fifth week after complete necrosis caused by fatal doses of sublimate in the recent group The regeneration starts very early, it may be observed already from the second day onwards and progresses with unequal speed in the individual cases, depending probably upon the general state of the patient and upon the success of the treatment The cell multiplication occurs partially by amitosis and multinuclear, flat elements develop, their numbers reaching a maximum during the second and third week, and decreasing later During the first weeks these cells exhibit also macrophagic properties, encircling necrotic substances and desquamating together with them from the basal membrane into the lumen of the tubule During the second and third week the epithelium gradually becomes differentiated into cuboidal epithelium, whose height increases in the late phases of this process, showing also a brush border Allen (1) states, that this epithelium is less sensitive to further sublimate poisoning

The question where the regenerating cells develop from is very interesting, appearing simultaneously underneath the completely necrotic epithelium of a very long tubule It is usually assumed that they develop from residues of the epithelium not affected by necrosis, nevertheless no undamaged nuclei may be found in the proximal tubules in the majority of cases of more severe poisoning In man, the short juxtamedullary part of the proximal tubule may be preserved in some cases ((1) and our cases Nos 1, 12, 13, 17), in some experimental animals the proximal part of the proximal tubule may be more sensitive, in others the distal one (4) The degree of regeneration, however, is the same in the whole cortex, so that it is difficult to consider the possibility of overgrowth of regenerating cells from the surroundings The prospective potency of the renal blastoma permits the idea of differentiation of activated interstitial elements in the direction towards the epithelium, but at present no proof is available in favour of this hypothesis

The *hydropic degeneration* of the epithelium in regenerated or preserved cells of the proximal tubules in our cases may probably be ascribed to therapy with hypertonic glucose Coarser vacuolisation could in some cases be considered to be a manifestation of dyspotassaemia (6) Fatty degeneration of the epithelium and more marked hyaline droplet formation do not belong to the picture of sublimate nephrosis

The occurrence of areas of *calcification* in the sublimate kidney is usually emphasized as it is quite regularly found in rodents — the most frequently used experimental animal But already Prévost (10) in 1882 noted the varying extent of calcification in various animal species According to our findings sublimate nephrosis in man in most cases is devoid of calcifications, the ratio of negative to positive cases being 15 7 The presence of calcifications in man neither depends on the dose of the poison, nor

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"Idiopathic" Hypoparathyroidism

Partial Gastrectomy as an Aetiological Factor?

By

Arne Thoren

The most usual form of hypoparathyroidism is the postoperative Hypoparathyroidism may also be caused by tumours syphilis X ray treatment, or may be congenital or an element in a malabsorption syndrome It may also appear as part of an endocrinopathy the combination as described particularly in association with Addison's disease (11 a o) Finally there is "idiopathic" hypoparathyroidism when the cause of the disease is unknown The more our knowledge increases, the smaller will be the number of "idiopathic" cases

Case report

A 57 year-old male hospitalized on April 6 discharged on April 30 1960 He had had gastritis since the age of 14 later developing into a duodenal ulcer He had had no abdominal trouble since a partial gastrectomy 5 years earlier Otherwise he had been previously healthy In February 1960 he had influenza (fever and general malaise) for 3—4 days followed by watery diarrhoea for a week and chilliness He consulted a physician and was given a vitamin injection and oral tonic On the following morning, 10 days after the onset, he woke normally, remained in bed and suddenly lost consciousness for the space of one hour A physician was summoned who considered the condition to be a typical calcium seizure (as evidenced by the position of the fingers) and gave him calcium injections intravenously, whereupon the cramp disappeared and the patient temporarily awoke He had no memory, however, of the course of events He soon became unconscious again was given new injections calcium and cardiac stimulants (probably nicetamide) and woke up again Thereafter and until his admission to hospital 4 weeks later he was given 12 calcium injections and gradually improved so was not given calcium the last 8—10 days He had constant nervous shivers however mostly in the epigastrium sudden onsets of nausea and vomiting felt "very low", displayed frequent trembling of arms and legs occasional numbness in arms hands and legs, was fatigued could only be up for a couple of hours at a time and lost 5 kg in weight.

He was admitted to this hospital six weeks after the first illness He was clear in his mind his general condition being as reported above The following examinations among others were made ESR 55 → 25 Fasting blood sugar 88 mg%, Blood picture normal Blood pressure 120/75 ECG normal Basal

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been shown by chemical and isotopic studies (11), whereas it increases in the kidneys. The mercurial deposits are removed from the kidneys very slowly, so that in the rat for example, 21 % of the administered dose may be still demonstrated in the kidneys 30 days after administration.

Chemical examination of the kidneys in anuria of uncertain origin is always of enormous importance considering the fact, that the histochemical proof of heavy metals (15) requires special fixation in alcohol saturated with hydrogen sulphide and also considerable laboratory experience and that the histological changes are in no way pathognomonic in the late phase of regeneration.

Summary

Morbid anatomical findings in 11 patients dying after poisoning with soluble mercurial salts during recent years have been compared with 11 cases from an older period. The influence of longer survival, depending upon treatment with the artificial kidney, has been evaluated with regard to the morphological picture of the poisoning. The findings of necrosis and regeneration in the proximal tubule is important for the histological diagnosis, calcifications, however, are not regularly found. From the fourth week of the disease onward the anatomical lesions in the kidney start to return to normal, but chemical examination still renders positive results. Even persisting pseudomembranous inflammations of the intestinal tract, especially of the colon may be of diagnostic value.

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tissue Atrophy of the parathyroid glands has been described (9, 15, 16) In one of these cases (9) the cause may have been a cerebral insult But it is difficult to imagine that unconsciousness would cause necrosis of normal parathyroid tissue Nor does the selective elimination solely of the parathyroid function tally with this explanation Finally the position of the fingers during the patient's first loss of consciousness and the immediate effect of calcium intravenously suggests that hypocalcaemia was present before the loss of consciousness

It thus appears more probable that, prior to the onset the patient had hypocalcaemia with latent tetany which was precipitated and aggravated by the acute illness The question is then what can have caused his hypocalcaemia

Some authors have intimated a relation between hypoparathyroidism and sprue (2 8, 16) One case of hypoparathyroidism has been described with findings typical of sprue in the intestinal X ray but without diarrhoea (12) In such case the hypocalcaemia would be part of a syndrome of malabsorption Judging from the past history and ordinary clinical examination of our patient he had no such *syndrome* but a special study was *not* made of this eventuality In this connection one must also consider the magnesium metabolism It has been demonstrated that the calcium and magnesium concentrations in the body economy are regulated by the same mechanism (10) and cases with postoperative hypoparathyroidism have been reported which have defied treatment with calcium and vitamin D but have promptly responded when magnesium was administered at the same time (6) Admittedly no tests for magnesium were made on our patient, but a magnesium deficit has been found only in states of intense and prolonged diarrhoea (14) and can hardly have existed in the present case The patient's diarrhoea was of short duration and associated with the acute phase of the illness and should be regarded as a part of the acute phase symptoms Neither before nor since has he shown signs of disordered intestinal function Nor was there any dietary defect

In 1961 Berlin and Rigner reported a case of 'idiopathic' hypoparathyroidism (3) Their patient like the present one had been gastrectomized Harvald et al (5) studied the calcium metabolism of gastrectomized patients and found signs of *deficient* calcium absorption in many of them Deranged calcium absorption and signs of calcium deficit have been observed previously in gastrectomized patients (e g 4)

The present patient had been gastrectomized five years before developing hypoparathyroidism He may in the meantime have had poor calcium absorption, in which case he would have low serum calcium but not so low as to cause tetany Calcium absorption takes place chiefly in the colon The acute infection accompanied by diarrhoea may have impaired his calcium absorption still further so that the tetany became manifest Something else must have occurred however otherwise the tetany should have disappeared when the intestinal function became normal or should at all events not immediately have become so grave as to require a very

nitrogen 25 mg%. No albuminuria or glycosuria. Thymol, GOT, GPT, OCT, aldolase, alkaline phosphatases, all normal. Electrophoresis revealed hypoalbuminaemia and moderately elevated α_2 and γ (as after acute infection). Normal urinary excretion of 17-ketosteroids, 11-hydroxysteroids, noradrenaline and adrenaline. Electrolytes in serum normal (except Ca and P). X-rays of skull, thoracic and lumbar spine, hand skeleton and lungs normal. No cataract. Water loading tests: specific gravity range 1008—1017, 790 ml excretion in 4 hours.

On admission Ca/s was 5.6 mg% and P/s 6.5 mg%. In the immediately succeeding weeks the patient was given 10 ml of calcium gluconate intravenously each second or third day without appreciable result. After two weeks Ca/s was 6.2 mg% and P/s 5.0 mg%. During the same period the urinary excretion of calcium and phosphorus was measured on seven occasions. Ca/u was constantly 0.01 g/day, P/u varied between 0.3 and 0.7 g/day, TRP was during the period 93, the intravenous calcium infusion test (15 mg/kg body weight/4 h) showed during the day of the test a calcium excretion of 0.07 g and phosphorus excretion of 1.0 g as against 0.01 and 0.34, respectively, during the control day.

After large doses of vitamin D_2 (Fortedol spirituosum[®]) had been administered the phosphorus and calcium levels in serum became normalized, though slowly (after at least a month) and only after very large doses (90,000 IU per day). Thereafter the patient had normal values, but on quite high doses of vitamin D. Since the high initial dose he has had 58,500 IU of vitamin D_2 daily during almost the entire time since his discharge from hospital. Several attempts to lower the dose have resulted in recurrent symptoms of tetany and deranged calcium and phosphorus in serum.

Discussion

The pathological picture agrees closely with that of hypoparathyroidism: nausea, vomiting, fatigue, malaise with "shivering" in epigastrium, trembling, numbness. Diarrhoea has also been reported as a not unusual symptom of hypoparathyroidism (1, 2, 16).

The patient appears previously to have been healthy apart from duodenal ulcer, and he had been free from stomach trouble since a partial gastrectomy 5 years previously. His hypoparathyroidism had a sudden onset without previous sign of failing parathyroid function and, judging from the high dose of vitamin D_2 needed, his parathyroid function appears to be entirely or almost entirely eliminated. There had either been an acute destruction of his parathyroid glands or he had a latent tetany which was precipitated and aggravated by the acute disease.

An acute destruction of the parathyroid glands may in this case be due either to toxic action or to disturbance of the blood supply.

The acute illness during the days prior to the first attack of unconsciousness manifested common symptoms which most people have repeatedly during their life. Furthermore the supposed toxic agent would act specifically on the parathyroid glands, since no sign of damage to the function of other organs was found either when the patient was examined in hospital or later. A toxic action is therefore hardly probable.

An acute necrosis of the parathyroid glands owing to failing blood supply is conceivable in conjunction with the patient's unconsciousness. Necroses have been reported in parathyroid adenomas, even with spontaneous healing of hyperparathyroidism as a result (7). But this was in a case of pathologically changed

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The Tolbutamide Tolerance Test

By

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The oral glucose tolerance test (GTT) has merely a limited value in the diagnosis of diabetes mellitus because it gives only a non specific expression of pancreatic function and may give pathological values in other disturbances of carbohydrate metabolism Tolbutamide stimulates the beta cells of the pancreas to secrete insulin and will in normal individuals give a rapid decrease in blood sugar In diabetics a smaller response is obtained or none at all and thus a latent diabetes mellitus may be unveiled This is the basis for the tolbutamide tolerance test (TTT) In order to obtain a rapid and maximal effect uninfluenced by the absorption from the gastro-intestinal tract, tolbutamide is given intravenously The present study records our experiences with TTT

Methods and materials

The procedure for TTT followed that of Unger and Madison (3) After determination of the fasting blood sugar 1 g of sodium tolbutamide dissolved in 10 ml of sterile water was given intravenously over a 2-minute period Blood samples for sugar determinations were taken 20, 30 and 60 minutes from the midpoint of the injection In almost all individuals studied 90- and 120-minute values also were determined Thirty five individuals were studied aged 15 to 81 years Thirteen were non-diabetics according to common criteria 6 were diabetics with the stable type of diabetes whereas 16 patients suffered from various diseases (table I) A standard GTT was performed in 26 individuals the minimum time interval between the two tests being 2 days The blood sugar was determined by the method of Hultman (4)

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Results

The results obtained in the 13 non diabetic individuals and in the 6 diabetics are shown in fig 1 In the non diabetics the blood sugar decreased distinctly after 20 minutes and a further fall was obtained after 30 minutes Thereupon the blood sugar increased slowly, reaching almost pre test values within 120 minutes

high dose of vitamin D_2. Here, possibly, the previously discussed circulatory disorder may have entered into the picture. Howard et al. (7) found necrosis of pathologically changed parathyroid tissue. If difficulty in maintaining calcium absorption sufficiently high had existed in our patient during a period of five years, it is reasonable to presume some degree of secondary hyperparathyroidism with hypertrophic parathyroids which might become necrotized as a result of the patient's repeated attacks of unconsciousness, the latter being undoubtedly due to a fall in blood pressure. The fact that the attendant physician gave cardiac stimulants in dealing with the second attack of unconsciousness may indicate a serious condition of shock.

Summary

A 57-year-old male patient is presented who, five years after partial gastrectomy, had symptoms of hypoparathyroidism, starting with sudden onset of unconsciousness. The pathological calcium and phosphorus levels became normalized slowly and only after large doses of vitamin D_2 (90,000 IU per day). It was thereafter necessary to give a daily maintenance dose of 58,500 IU.

It would seem that hypoparathyroidism must now be borne in mind as a late complication after partial gastrectomy.

Partial gastrectomy may possibly be one of the aetiological elements in "idiopathic" hypoparathyroidism.

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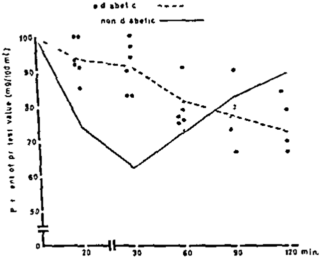


Fig 1 Six patients with manifest diabetes mellitus diabetic symptoms persistent glucosuria and fasting blood sugar > 120 mg/100 ml (The 90 and 120-minute values were determined only in 5 of them) Thirteen non-diabetic individuals no diabetic symptoms no glucosuria fasting blood sugar < 110 mg/100 ml GTT of non-diabetic type (The 90- and 120-minute values were determined only in 12 of them)

time all values in the non diabetic group had decreased by more than 25 per cent of the pre test value In the diabetic group however no 30-minute value had fallen by as much as 25 per cent of the pre test value Consequently the30 minute value was regarded the most reliable in the diagnosis of a diabetic or non diabetic state A blood sugar fall of less than 25 per cent indicates a diabetic response a 25 per cent fall or more a normal response

According to this criterion 16 patients with various diseases were divided into 3 groups (table I) The patients in group A had either diabetic symptoms glucosuria or elevated fasting blood sugar In this group both GTT and TTT were consistent with a diabetic state Group B showed no conformity of the two tests In group C the patients had glucosuria on admission disappearing later Three patients showed a little elevated fasting blood sugar values which after a few days returned to normal In these patients TTT was normal and they were consequently classified as non diabetics GTT was omitted

Fig 2 gives the 20 and 30-minute values for all the individuals studied

Discussion

The diagnostic criteria used by Unger and Madison (7) were A blood sugar fall of 20 per cent or more from the pre test value in 20 minutes indicates a normal response a fall of less than 15 per cent indicates a diabetic state If borderline values (i e 15—19 %) is obtained at 20 minutes, a 30 minute fall of 25 per cent or more is required to be regarded a normal response In the present study 6 patients had a 20-minute value between 15 and 19 per cent From fig 2 it will be seen that 3 of these patients at 30 minutes showed a diabetic response whereas the other 3 showed a normal blood sugar fall at 30 minutes In no patient did the 30-minute value

TABLE I

Pat No	Sex, age	Diagnosis	Diabetic sympt	Glucosuria	Bl sugar (mg/100 ml)	TTT % fall from pre-test blood sugar value after 20, 30, 60, 90 and 120 minutes		GTT Blood sugar pre test values, peak values time in hours when pre test value regained	
						Non diab result	Diabetic result	Non-diab result	Diabetic result
Group A									
1	♂ 32	Acromegalia	–	–	114		12—22—15—14—8		105—185 3
2	♀ 58	Cholelith	–	+	115		4—8—10		95—208 3
3	♀ 50	Mb Cushing oper	–	+	130		15—20—37—38—30		110—273 2 1/2
4	♀ 30	Anorexia nervosa	–	+	112		12—23—38—35—20		122—261 2 1/2
5	♀ 33	Diabetes insipidus	+	–	90		11—23—37		90—240 2 3/4
6	♀ 68	Ca pancr	–	–	127		0—0—3—1—4		120—238 3
Group B									
7	♀ 15	Malabsorpt syndrome	–	–	100		9—18—9—2—0	88—138 2 1/2	
8	♂ 45	Malabsorpt syndrome	–	–	92		0—8—11—0—0	92—117 2 1/2	
9	♂ 62	Nephr chr	+	–	97		12—18—37	92—174 2 1/2	
10	♂ 54	Alch chr	+	+	117		8—21—41	82—177 1 3/4	
11	♀ 63	Ca hepatis	–	–	101		18—22—51—48—37	105—134 2 1/2	
12	♂ 28	Hepatitis chr	–	+	80	35—42—32 16—8			78—262 3
Group C									
13	♂ 65	Inf cordis	–	+	103	26—38—33			
14	♂ 72	Inf cordis	–	+	115	21—31—22			
15	♂ 81	Inf cordis	–	+	117	27—47—33			
16	♂ 29	Pneumonia	–	+	128	23—31—28			

In the diabetics the response was poor and slow A small decrease was obtained after 20 minutes The values were almost unchanged after 30 minutes, whereas later a somewhat higher response was obtained The normal and the diabetic group showed distinctly different values after 30 minutes At this

TTT in these patients indicates a latent diabetes mellitus not revealed by GTT

It is well known that patients suffering from extensive myocardial infarction and severe lobar pneumonia may have glucosuria and elevated fasting blood sugar, probably an expression of a stress reaction The TTT performed in 4 such patients (group C) shows that they were not diabetics

In the present study 5 non diabetics complained of being hungry at the end of the test, and a single patient experienced nausea some 5 minutes after the injection Another felt uneasy after about 50 minutes his skin was a little clammy, but the pulse and blood pressure were normal The blood sugar at this time was 70 mg/100 ml

Because some patients may experience an aching pain along the vein during the injection the tolbutamide should be dissolved in 20 ml of sterile water and the injection time prolonged to 3 minutes

An important indication for TTT is insuloma where there occurs a blood sugar fall considerably greater than normal and even greater than that obtained in individuals with functional hyperinsulinism (2 6) A pronounced hypoglycemia may appear during the test necessitating glucose infusion

As to the decrease of the blood sugar by TTT in relation to insulin lack no distinct correlation seems to exist (3) In general however the more severe the diabetes the less the response to tolbutamide Thus in juvenile diabetes no insulin will be released (5)

In conclusion TTT seems to be a valuable diagnostic tool for the evaluation of carbohydrate metabolism In some patients it may be used as a supplement to GTT, in others it may replace it It is not time consuming, and if used as a screening test it entails only two blood samples, one fasting and one at 30 minutes This is important both for the patient and for the laboratory The injection must be done by the doctor, but supervision by him during the entire test seems unnecessary except when insuloma is suspected

Summary

Thirty five individuals between 15 and 81 years were examined for their response to the intravenous tolbutamide tolerance test (TTT) In 26 an oral glucose tolerance test (GTT) was also performed, with a minimum time interval of 2 days between the tests It is concluded that

a) TTT is a valuable diagnostic tool for the diagnosis of diabetes mellitus
b) Only 2 blood sugar determinations are required one fasting and one at 30 minutes

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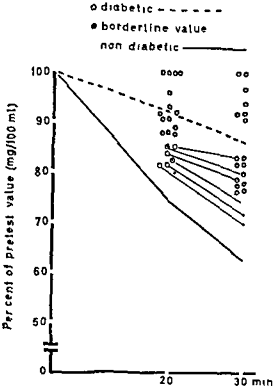


Fig 2 The blood sugar values at 20 and 30 minutes for all individuals studied The 6 borderline 20 minute values (after the criteria of Unger and Madison) are specially marked, showing a fifty fifty distribution between the diabetic and the non-diabetic group at 30 minutes

show a diabetic response and the 20-minute a non-diabetic Likewise, in no case did a normal 30 minute value coincide with a diabetic 20 minute value This supports the validity of the diagnostic statement given above a blood sugar fall of less than 25 per cent at 30 minutes indicates diabetes mellitus

Concerning the patients in group A, table I, most of them had some but none of them all signs indicating diabetes mellitus (see criteria in the text for fig 1) They were therefore not included in the group 'diabetics", even if the GTT indicated a diabetic state The TTT gives an additional proof of the diagnosis in the two first patients, and also in patient No 3 who developed the disease after adrenalectomy The carbohydrate metabolism in patient No 4 probably was disturbed by the starvation, the glucosuria, the elevated fasting blood-sugar and the results of both GTT and TTT indicate a diabetic state This shows that in anorexia nervosa also insulin secretion might suffer The diagnosis of diabetes insipidus in patient No 5 was established by the results of the water restriction test, the vasopressin test and the Hickey Hare test The concomitant presence of diabetes mellitus is uncommon, but is here indicated both by GTT and by TTT The results in patient No 6 are in agreement with the report of Berkowitz (1), who on examination of 18 patients suffering from cancer of the pancreas found a diabetic response in 11 by GTT and in 13 by TTT

The two patients in group B suffering from a malabsorption syndrome showed a diabetic TTT response Both had low fasting blood-sugar values when TTT was performed, 60 mg/100 ml This might explain the diabetic TTT response even normal beta cells are unable to release sufficient insulin when the initial value is low, and thus the result wrongly will be diabetic In patient No 12 the discrepancy between GTT and TTT is noteworthy Due to the hepatic derangement GTT is of diabetic type, whereas TTT shows a normal pancreatic function In the remaining 3 patients in group B the discrepancy between the two tests probably is an expression of the diagnostic limitation of TTT a diabetic result is obtained in non diabetic individuals Admittedly, the possibility exists that

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The M-Value, an Index of Blood-sugar Control in Diabetics

By

J SCHLICHTKRULL, O MUNCK and M JERSILD

Clinical assessment of the diabetic state during treatment naturally carries some uncertainty due to the lack of a method for evaluating the differences of the various data from normal (hyper and hypoglycaemia, glucosuria acidosis etc) A more precise method of assessment would be useful, e g in evaluating the various methods of treatment and in characterizing the lability of diabetes

The present study attempts to afford an objective appraisal of the quality of the therapeutic results On the basis of blood sugar data and a simple formula a numerical value, called the M value, is calculated This value is a quantitative index of the lack of efficacy of the treatment in the individual patient

Previous investigations

The first major systematic clinical study of a slow acting insulin was performed by Kramp in 1935 (5) His material consisted of in patients on a strict diet, and each insulin preparation was administered for periods ranging from 1—3 weeks The quality of the control was assessed on the basis of the mean blood sugar curves, the glycosuria, and the excretion of ammonia The question posed was whether protamine insulin was more effective than regular insulin administered once or twice daily The results were so clear cut that a statistical analysis was unnecessary

Izzo and Crump (3) in 1950 *tried to* compare and evaluate the *effects of* various slow acting insulins This is of course more difficult since the differences *are not* as marked as between protamine insulin and standard insulin On the basis of 4 BS (blood sugar) and 4 US (urinary sugar) values in 24 hours, they calculated the variations within the 24 hours as well as the day to-day *fluctuations* The statistical estimates were useful in the classification of patients according to stability of blood sugar *but they* could not be used as a *measure for* the quality of control and

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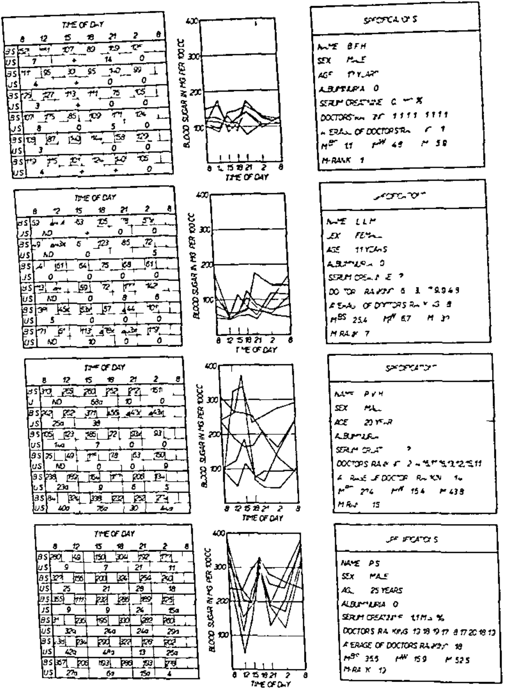
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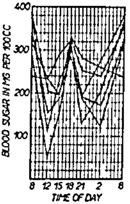
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Symbols ΔD no diuresis + = trace of sugar 0 = no sugar a = acetonuria Δ = insulin sensation x = ingestion of additional carbohydrate

Fig 2 Four sets of data classified in the same order in all nine quality rankings

	TIME OF DAY 8	12	15	18	21	2	8
BS	280	149	150	304	192	177	
US	9		7		21	11	
BS	322	156	200	324	254	240	
US	25a		21		28	18	
BS	365	171	232	286	189	175	
US	9		9		24	15a	
BS	312	236	195	330	282	280	
US	32a		24a		24a	29a	
BS	238	234	290	322	136	202	
US	42a		48a		13	76a	
BS	367	206	193	286	193	278	
US	27a		6a		15a	41a	



SPECIFICATIONS

NAME PS
SEX MALE
AGE 25 YEARS
ALBUMINURIA 0
SERUM CREATININE 1.1 MG %

Fig 1 One of the twenty sets of clinical and laboratory data used for the evaluation of the M formula (a = acetonuria)

thus serve to determine the differences between the efficacy of the preparations

In 1954 Hallas-Moller et al (2) published a paper on the Lente Insulins Each preparation was administered for about one week to in-patients on a fixed diet, and the BS was determined 6 times in the 24 hours Three parameters were calculated for each patient and period (1) The mean of all BS values, (2) the range, Δ BS, between the maximum and minimum value in the mean 24 hour blood sugar curve, and (3) a measure of the dispersion of the individual 24-hour blood sugar curves The Δ BS-value was used to indicate the quality of the adjustments except in that part of the series in which the dispersion was high However, like the other two parameters, Δ BS cannot be accepted as a general index of quality

Jersild (4) assessed the adjustments by counting how large a percentage of the blood sugar values were in the range $75 < BS < 200$ mg/100 ml If the percentage was 80 or over, the patient was considered well controlled This measure (the percentage) is consistent and objective, but it is doubtful whether it agrees in all cases with the clinical estimate

In a large series of out patients Gurling et al (1) determined the quality of the control on the basis of the sugar content in the morning urine, BS before lunch, and fluctuations in body weight Such an investigation into the control in ambulatory patients falls outside the scope of the present study which deals with the immediate therapeutic results obtained in hospital patients

Present investigations

The study was designed to find an objective, reliable, and sensitive index of the quality of control and thereby of the efficacy of the treatment or the insulin used Such an index presupposes a thorough description of the therapeutic results, e g by frequent blood sugar determinations, and this can be done only when the patients are in hospital

The observed data *per se* are inapplicable as an index of quality (if only because there are many data for each patient) The object was then to set up

TABLE II Calculation of the M value from 36 BS values (in brackets) in an experimental period of 6 days

Time of day / Day	8	12	15	18	21	2	Sum
1	(59) 28	(47) 77	(63) 19	(105) 0	(78) 6	(57) 40	170
2	(49) 55	(43)* 77	(61) 28	(123) 0	(86) 4	(72) 13	177
3	(84) 4	(61) 28	(64) 19	(76) 9	(68) 13	(61) 28	101
4	(113) 0	(49) 55	(59) 28	(72) 13	(177) 4	(142) 0	100
5	(138) 0	(45) 77	(53) 40	(57) 40	(44) 77	(101) 0	234
6	(171) 3	(51) 55	(113) 0	(78) 6	(53) 40	(119) 0	104
						Total	886

$M^{BS} = 886/36 = 25$
$M^{W} = (177^{*} - 43^{*})/20 = 7$
$M \;\; = 25 + 7 = 32$

tion of the course of the blood sugar the 24 hour M value is calculated as the mean M^{BS} of M values called M^{BS}_{BS} calculated for the various blood sugar data of the 24 hours

4 The M^{BS}_{BS} value is to measure the lack of efficacy of the treatment at the time when the blood sugar concerned was determined Let M^{BS}_{BS} be defined tentatively as the difference between the observed blood sugar and normal blood sugar 100 Then for BS = 40 and BS 160 M^{BS}_{BS} is in either case 60 At a near normal BS of 160 however the control must be considered more effective than at BS = 40 at which hypoglycaemic symptoms presumably occur Thus the primitive formula $M^{BS}_{BS} = |BS - 100|$ is not suitable Instead we suggest

$$M^{BS}_{BS} = \left| 10 \times \log \frac{BS}{120} \right|^{3} \quad (1)$$

which is illustrated in table I

The suggested formula was tested on the 20 sets of data and the test gave a *ranking* which accorded equally well with the mean sequence as the rankings made individually by the various physicians

However it was possible to get even closer As might be expected the physicians evaluations paid regard not only to the abnormal blood-sugar values but also to the *occurrence* of major blood sugar fluctuations which are not considered in the suggested formula The formula was therefore corrected by adding a term M^{W} W/20 for *fluctuations* in which W = the difference between the maximum and minimum blood-sugar value during the period concerned Thus the final M formula was

TABLE I Table of M_{BS}^{BS} values corresponding to BS values from 15—595 Examples (1) BS = 45, $M_{BS}^{BS} = 77$, (2) BS = 320, $M_{BS}^{BS} = 75$

BS	0	100	200	300	400	500
0	—	1	11	65	140	240
5	—	0	13	65	145	245
10	—	0	14	70	150	250
15	740	0	16	75	155	250
20	470	0	18	75	160	260
25	320	0	20	80	165	260
30	220	0	22	85	170	270
35	150	0	25	90	175	270
40	110	0	27	95	180	280
45	77	1	30	95	185	280
50	55	1	32	100	190	290
55	40	1	35	105	195	290
60	28	2	38	110	200	300
65	19	2	41	110	205	300
70	13	3	44	115	210	310
75	9	4	47	120	215	320
80	6	5	50	125	220	320
85	4	7	53	130	225	330
90	2	8	56	135	230	330
95	1	9	60	140	235	340

a formula which when applied to the data gave the desired measure The formula, which is to imitate the clinical assessment, could not be deduced from existing information, since there are insufficient descriptions of how the clinical evaluation is made and insufficient published examples of its use Accordingly, there was only one possibility To establish a number of therapeutic results, evaluate the data clinically, and set up a formula which when applied to these data would give a measure in accordance with the clinical assessment

Twenty sets of data from 20 insulin treated patients with widely different therapeutic results were selected from a larger series The data of one set is exemplified in fig 1

Nine persons, familiar with insulin therapy, were asked to rank, independently of each other, the 20 sets according to their judgment of the quality of therapeutic results (No regard was paid to the quality of the *treatment* — whether the results might have been better — since this is a different matter) The 20 sets of data were ranked in 9 different forms of sequence As might be expected there were similarities in the evaluations as shown in fig 2 All the persons ranked these four cases in the listed sequence

As a final integrated evaluation of the 20 therapeutic results, the 9 rankings were combined in one mean ranking which the formula had to reproduce as far as possible

The next step was to make up a formula which then had to be tested against the mean ranking and possibly improved The following reasoning was considered

1 The formula must, for each set of data, give a value called the M value, which measures the treatment's lack of efficacy Therefore, M is zero in normal or normalized persons and the value is higher the poorer the control of diabetes during the treatment
2 All days of the observation period (usually 6 days) are of equal importance in evaluating the therapeutic result, since the period does not *start until the treat*ment has resulted in such a degree of stability that the data do not show systematic day to-day alterations Therefore the M value for the result and the period has to be calculated for each day of the period
3 The M value for each individual day is calculated on the basis of the blood sugar curve which is assumed to be the most important criterion Blood sugar values from all hours are considered equally important and as the 6 specimens fairly equally distributed over the 24 hours afford a representative descrip-

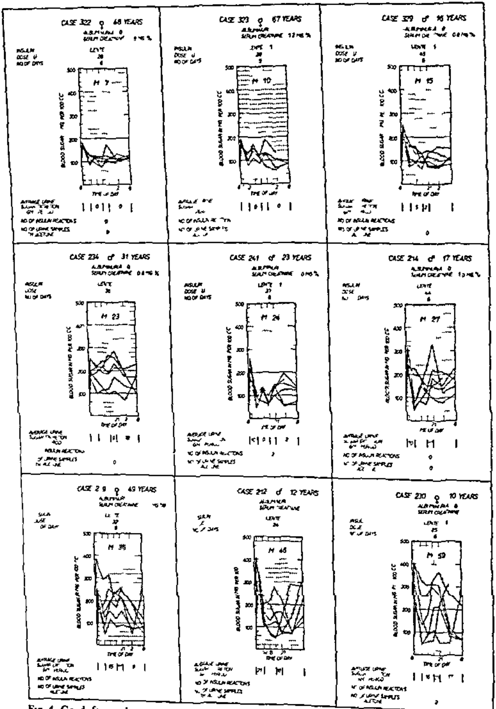


Fig 4 Good fair and poor control

TABLE III Distribution of patients according to the M value

M value corresponding to 7 daily BS values	Percentage distribution in 413 consecutive insulin treated diabetics
0—18 (good control)	28
19—31 (fair control)	42
32— (poor control)	30

$$M = M^{BS} + M^{W} \quad (2)$$

in which M^{BS} is the average of all M_{BS}^{BS}-values (formula (1)) Now, the formula could not be improved further, neither by inserting corrections for glycosuria aceto nuria, nor those for the occurrence of insulin reactions The calculation of the M value on the basis of table I is exemplified in table II

On a given treatment the result and the M value in the same patient will vary somewhat from one period to the next The coefficient of variation for the M value was found to be approx 15 % for 6 day periods both for high and low M values

The M value in normals was calculated from Trimble and Maddock s (8) data from nine normal young men on a fixed diet The M value was between 1 and 4 (fig 3)

For the purpose of illustrating the relationship of the M value to observed data fig 4 sets out examples of a good fair and poor control as defined below

Routine blood sampling for determining the blood sugar at 9 a m was introduced because as a rule the blood sugar is at a maximum at this hour The 9 a m BS determinations are included in the calculation of the M values in fig 4 The M values for 7 daily BS values were found to be on the average 23 % higher than with only 6 determinations The reliability of the M formula increases with the number of daily BS determinations If the number of determinations is reduced to less than 6 in the 24 hours the formula may possibly be improved by inserting corrections based on the gly

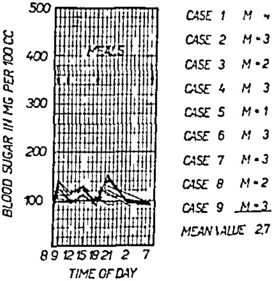


Fig 3 BS curves and M values in 9 normal subjects (from the data of Trimble and Maddock (8))

cosuria and the occurrence of hypoglycaemic symptoms

When a patient has attained the best possible ' control in hospital the corresponding M value may be said to measure the lability of the diabetes On the basis of data from 413 consecutive insulin treated patients admitted in 1960 the M values for the best periods were calculated The 413 M values were classified by size into three approximately equal groups as shown in table III

Accordingly the control was defined as good in the group having the lowest M values ($0 \leq M \leq 18$) fair with $19 \leq M \leq$ ≤ 31 and poor with $32 \leq M$

Discussion

The M formula has been used with satisfaction in the hospital for three years It has been used in comparing various insulin therapies (7) and in statistical analyses on the distribution of lability in a patient material in relation to age, sex duration of diabetes and late diabetic complications (6)

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Insulin Rapitard and Insulin Actrapid

By

J Schlichtkrull, O Munck and M Jersild

In insulin therapy a distinction is made between three types of insulin preparation (1) ordinary, quick acting insulin, (2) very slow acting insulin (protamine zinc insulin (1, 18) and Ultralente (3, 4)) and (3) preparations (e g NPH (7) and Lente (5)) having an intermediate action In the treatment by one or two injections per 24 hours the intermediate effect is usually preferred since soluble, ordinary insulin (S I) does not act for a sufficient length of time and P Z I or Ultralente exert too weak an action during the hours after the morning injection (8) Frequently, the intermediate effect too is initially too weak and it is therefore supplemented by regular quick acting insulin It seemed reasonable to try to combine these two types of insulin in one preparation A modified protamine zinc insulin with a 25 % content of dissolved ordinary insulin was studied clinically and suggested for general use (9—11) but as the preparation proved unstable (12) the idea was abandoned

During recent years the idea of such a combined preparation has been resumed (14, 15) and realized (16) in the form of Insulin Rapitard which is a suspension of slow acting crystals of pure insulin (30 i u /ml) in a neutral solution of quick acting, ordinary insulin (10 i u /ml) The quick acting component is also available as a separate preparation Insulin Actrapid, which is ordinary, soluble insulin having a neutral reaction (pH 7 4)

In 1940 it was demonstrated by Sahyun (13) in experiments on fasting rabbits that S I had an immediate effect on the blood sugar when administered subcutaneously in the form of a neutral (pH 7) solution, while the effect was delayed for about 10 minutes with the usual acid solution (pH 2 9) Sahyun explained the delay as the time required for the neutralization of the acid insulin solution in the tissue before its absorption Apparently no one else has reported on the production or use of S I with a neutral reaction, presum

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The general applicability of the formula is restricted by the presupposition of an adequate representation of the blood sugar which requires a number of determinations in the 24 hours The principle underlying the formula, the setting up of a mathematical substitute for clinical evaluation, may possibly be applied with advantage to other situations, e g the evaluation of the out-patient control The drawing of conclusions regarding a single case or a class of patients from the amorphous mass of accumulated data might be greatly aided through formularization and statistical treatment

Summary

A value, called the M-value, was calculated, by means of a simple formula, on the basis of the blood sugar data The M-value is a quantitative index of the lack of efficacy of the treatment in the individual diabetic patient The formula, which is an objective substitute for clinical evaluation, was set up in an attempt to obtain a mathematical imitation of the integrated clinical estimate made by 9 physicians of 20 different therapeutic results The significance of the M-value is illustrated by clinical examples in which the formula is used for assessing various treatments and for characterizing the lability of the diabetes

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sulin Insulin Actrapid is a neutral solution of porcine insulin

Table I lists the composition of various insulin preparations

In clinical practice the insulin preparations are often mixed (cf table II) in the syringe

In mixtures of Rapitard and Actrapid the components do not modify each other, but preserve their characteristic properties and actions Like other ordinary insulin, Actrapid reacts chemically when mixed with P Z I , these mixtures exert an intermediate action On the other hand, the initial action is preserved if Actrapid or another S I is mixed with NPH insulin in the injection syringe Actrapid may also be mixed with Lente or Ultralente in the syringe This usually gives a somewhat stronger insulin effect than mixture with Semilente during the first several hours after the injection

The insulin crystals in Rapitard are identical (except for a low content of zinc) with the crystals in Ultralente and Lente When shaken Rapitard and Ultralente in particular show peculiar reflections of light schlieren caused by unification of the orientations of the crystal surfaces and not by fractionation or lack of homogeneity of the suspensions The standard deviation in the administration of 40 i u insulin by a 1 ml syringe has been found to be 2—3 % with Rapitard as well as Actrapid (The insulin was determined by ultraviolet absorption measurements) The insulin suspensions must be shaken immediately before the injection is performed The filled syringe must not be left before injection as it has been shown experimentally that insulin crystals sedimented in the syringe may elude injection

TABLE III Diet as distributed on the meals

Hour	Carbohydrates	Fat	Protein	Calories
8 a m	60	18	14	475
noon	57	28	26	600
3 p m	21	11	6	225
6 p m	52	36	22	625
9 p m	12	9	5	150
Total	202	102	73	2 075

Methods

Clinical model

The present study was undertaken in an attempt to assess the efficacy in practice of various forms of insulin therapy using different insulins and giving one or more injections per day It is supposed that the material as well as the experimental conditions are representative in the sense that the results permit conclusion applying more or less universally It was impossible to get the material by random selection of patients from the insulin treated diabetic population The material consists of consecutively hospitalized patients excluding the stable cases (having a low M value (17)) who did not respond perceptibly (low Δ M value (*vide infra*)) when shifted from one insulin to another The morning injection was administered at 8 a m In cases on two-injection therapy the second injection was given at 5 30 p m The diet was distributed in 5 meals In table III a standard diet of approximately 2 000 calories is shown

Samples for determination of the blood sugar concentration were withdrawn at

TABLE I The composition of various U 40 insulins P Z I — Novo contains 35 mg of protamine/100 ml All the preparations contain 0 1 % methyl p-hydroxy benzoate per ml Isotonia is obtained with 5 % glucose in Insulin Novo, 1 6 % glycerol in P Z I — Novo and 0 7 % sodium chloride in the other preparations Insulin Novo contains no buffer, while the other preparations contain 0 01 M sodium acetate and show neutral reaction (pH 7)

	Species	Physical state	pH	*Concentration of Zn* (mg/100 ml)
Insulin Novo	Porc + beef	Solution	3	3
P Z I — Novo	Porc + beef	Amorphous	7	9
Semilente	Porc	Amorphous	7	9
Lente	Porc + beef	Amorphous + crystals	7	9
Ultralente	Beef	Crystals	7	9
Actrapid	Porc	Solution	7	1
Rapitard	Porc + beef	Solution + crystals	7	1

TABLE II Modification of insulin preparations by extemporaneous mixture in the injection syringe

Type of insulin	Slow acting component	May be modified by
P Z I	Amorphous protamine zinc insulin	Reg I [1], Actrapid[1]
NPH Leo Retard	Cryst protamine (zinc) insulin	Reg I Actrapid Cryst P Z I
Lente	Amorphous and cryst insulin zinc	*Actrapid*[1], *Reg I*[1] *Semilente* Ultralente
Rapitard	Insulin crystals	Actrapid (— neutral Reg I)

[1] The mixture acts as an intermediate preparation, not as a combination of separate injections of the two preparations

ably because of the complicated stability problems at pH 7 (12)

Chemistry

The absorption and action of insulin *may be prolonged by injecting sparingly* soluble insulin compounds, such as e g the amorphous or crystalline P Z I compounds (P Z I , NPH) and insulin zinc compounds (the Lente insulins) Insulins obtained from various species of animals do not exhibit exactly the same solubility at pH 7, and a neutral suspension of the relatively sparingly soluble ox insulin crystals has also proved to exert a prolonged action after subcutaneous injection (*15*) *Since* the suspension does not contain alkaline precipitants, such as protamine and zinc, it may also be made to contain dissolved, quick acting porcine insulin which is more soluble than beef insulin at pH 7 In Rapitard 75 % of the insulin is in the form of suspended beef insulin crystals and 25 % in the form of dissolved, quick acting porcine in

(gr = group) By calculating the standard error of ΔM_{gr} and applying the t test it is ascertained whether the observed difference is significantly different from zero Even though ΔM does not have the same sign in all patients of the group, and this is the general rule ΔM_{gr} is used nevertheless as a measure for the group just like ΔM for the individual patient A ΔM_{gr} value differing significantly from zero is used as a criterion for deciding which of the two treatments is better for the group The treatment which was found to be "better in this way also proved to show the lower M value in the majority of patients Accordingly there was no conflict between the two criteria ΔM_{gr} and the relative frequency (f) of patients showing the same ΔM sign as ΔM_{gr}

Example

Table IV gives a comparison of Insulin Rapitard and Soluble Insulin (S I) both administered in two daily injections in a group of 11 patients The average dose was 43 i u Rapitard daily, while the dose of S I had to be about 50 % higher to obtain optimum adjustment The difference in dosage is significant ($P < 0.1$ %) The table shows which treatment was used first Incidentally the sequence proved to have no perceptible influence upon the results of the comparisons Regardless of the treatment given the highest blood sugar value was usually found at 9 a m at which hour the blood sugar generally reached a maximum after the greatest postprandial rise To ascertain the difference between the efficacy of the two treatments at this hour we calculated $\Delta\overline{M^{BS}9} = \overline{M^{B}9}_{Rap} - \overline{M^{BS}9}_{SI}$ in which e g $\overline{M^{BS}9}_{Rap}$ is the mean $M^{BS}9$ value for the group treated with Rapitard $M^{BS}9_{Rap}$ is the mean of the individual patient s M^{BS}_{BS} values at 9 a m during the observation period on Rapitard

In the example given above there is a marked difference $\Delta\overline{M^{BS}9} = -81$ between the efficacy of the two treatments at 9 a m The average $\overline{M^{BS}9} = 32$ for Rapitard correponds to a blood sugar value of 250

TABLE V Distribution of patients according to their smallest M value

M value	Percentage distribution	
	Present series (114 cases)	413 consecutive cases
0—18 (good control)	9	28
19—31 (fair control)	55	42
32— (poor control)	36	30

(17, table 1) while for S I it corresponds to a blood-sugar value of 370 On the whole the control appears to be considerably poorer on S I twice daily Accordingly $\Delta M_{gr} = -21$ and is statistically significant from zero ($P < 0.1$ %)

To form an impression of the blood sugar behind a known M (and ΔM) the reader should consult fig 4 in the previous publication (17) e g examples $M = 24$ and $M = 46$ The quality-difference between the adjustment in these two examples is measured by $\Delta M = 22$ An impression of the clinical significance of ΔM may be gained from the distribution of M values in table III in the previous publication (17) Among 413 consecutively hospitalized patients 28 % were found to have an M value lower than 19 and 30 % an M value exceeding 31 In other words the ΔM value is 12 or over when a patient moves from the group of 30 % with the poorest adjustments to the group of 28 % showing the best control (or *vice versa*) If the cumulative distribution of the M values is tabulated or plotted this relationship may be read more accurately Clinical introduction of a treatment which lowers the M level of the best obtainable adjustment also alters the above mentioned M-distribution

It will be noticed that in table IV ΔM has the same sign throughout and that consequently the relative frequency f of patients who are "better on the treatment

TABLE IV Comparison of Rapitard insulin and S I (soluble insulin, regular insulin) both administered in two daily injections

n=11			2×Rapitard			2×S I					
Case	No of days per period	Insulin first given	Dose	M^{BS9}	M	Dose	M^{BS9}	M	Δ Dose	Δ M^{BS9}	Δ M
239	6	S I	37/12	11	45	37/31	108	61	—19	— 97	—16
240	6	S I	40/24	22	28	37/31	91	43	— 4	— 69	—15
241	6	S I	20/16	12	12	21/16	35	25	— 1	— 23	—13
242	6	S I	35/15	16	26	35/29	136	61	—14	—120	—35
246	6	S I	19/ 8	12	27	32/24	61	31	—29	— 49	— 4
247	6	S I	33/ 8	69	43	37/35	134	58	—31	— 65	—15
250	6	Rapitard	36/15	43	31	41/35	100	59	—25	— 52	—28
251	6	S I	32/ 6	37	38	35/14	145	57	—11	—108	—19
252	6	Rapitard	28/ 7	33	39	57/30	133	68	—52	—100	—29
254	6	Rapitard	26/12	27	30	49/25	138	62	—36	—111	—32
264	6	S I	26/ 9	62	35	39/16	156	57	—20	— 91	—22
Average			43 0	31 7	32 2	64 1	112 5	52 9	—22 0	—80 7	—20 7
S L									4 5	9 3	2 8
P %									< 0 1	< 0 1	< 0 1

f = 11/11 = 100% (72%—100%) (P = 5%)

8 a m, 9 a m, at noon, 3 p m, 6 p m, 9 p m, and 2 a m The urine was collected at 8 and 11 30 a m, 5 30 and 10 p m (The data from the urine analyses were not used in the present study)

Appraisal of data

In evaluating the therapeutic results we used the M formula described in a previous paper (17) The observed blood sugar data are inserted in the formula which affords a value, the M value, as a measure of the lack of efficacy of the treatment in a given case during the observation period If a patient is treated with two different insulins I and II the difference between the therapeutic results is calculated as the difference, $\Delta M = M_I - M_{II}$, between the two corresponding M values (M_I and M_{II}) using an observation period of 6 days for each treatment and a few days (2—5) for the readjustment The difference, ΔM, carries an uncertainty, because a patient differs in his response to insulin in the course of time, the 24 hour blood sugar curves on a given treatment differing more or less from one day to another Furthermore, ΔM carries an error if the patient s diabetes has been in an unusual e g particularly labile state during the observation period

The response to insulin differs individually so that ΔM will vary and perhaps change signs from one patient to another If ΔM has the same sign e g negative in all patients of a group all the patients have exhibited better control — lower M value — on insulin I which therefore must be said to have been more favourable in the group concerned A measure of the quality-difference between the two treatments tested on the group may be obtained by calculating the patients mean ΔM value ΔM_{gr}

Table VI (Cont)

n = 50			1 × Rapitard			1 × Lente					
Case	No of days per period	Insulin first given	Dose	M^{B59}	M	Dose	M^{B59}	M	Δ Dose	Δ M^{B59}	Δ M
453	6	Lente	41	24	27	44	44	28	−3	−20	−1
454	6	Rapitard	51	94	39	48	57	35	3	37	4
455	6	Rapitard	38	38	41	34	52	42	4	−14	−1
456	6	Rapitard	45	29	27	43	46	39	2	−17	−12
457	6	Rapitard	55	28	30	53	75	40	2	−47	−10
458	6	Rapitard	34	6	16	32	18	19	2	−12	−3
459	6	Lente	31	39	34	34	14	28	−3	25	6
461	6	Rapitard	40	34	20	40	61	31	0	−27	−11
463	6	Rapitard	34	17	26	30	42	40	4	−25	−14
Average			36 6	42 9	32 7	35 5	51 7	35 5	1 1	−8 9	−2 7
S E									0 4	3 6	1 1
P %									2	2	2

f = 31/50 − 62 (46 %—75) (P = 5 %)

which is better for the group as a whole, is 100 %. The table also states the statistical confidence limits (P = 5 %) of f (72 % — 100 %)

Material

The study comprises 114 diabetics 72 males and 42 females The average age in the entire series was 34 years (7—67) in the male group 35 years (7—67), and in the female group 33 years (8—66) In table V the distribution of the patients in respect to the M value of the best obtainable adjustment is compared with the distribution in a previous series comprising 413 consecutive insulin treated diabetics (17 table III)

Out of the 114 diabetics 95 were used for one comparison while 19 were used in two or more of the total of 136 comparisons

Results

One injection therapy

In a preliminary series a suspension like Rapitard but with a smaller content (8 %) of dissolved insulin gave equally good, if not better control than Insulin Lente An essentially greater content (40 %) of dissolved insulin makes the prolonged insulin action from the crystals too weak As shown in table VI, an unexpectedly good result was obtained by 25 % dissolved insulin i e by Rapitard

The mean M value as well as the M^{B59} value were lower on Rapitard which gave better control (lower M-value) in about two-thirds of the 50 cases In order to illustrate the comparisons the blood sugar curves for the first

TABLE VI Comparison of Rapitard insulin and Lente insulin both administered in one daily injection

n = 50			1 × Rapitard			1 × Lente					
Case	No of days per period	Insulin first given	Dose	M^{BS9}	M	Dose	M^{BS9}	M	Δ Dose	Δ M^{BS9}	Δ M
332	5	Lente	40	50	28	40	59	34	0	−9	−6
334	5	Rapitard	46	17	28	39	29	29	7	−12	−1
350	6	Rapitard	52	33	31	52	34	35	0	−1	−4
367	6	Rapitard	43	28	24	40	67	36	3	−39	−12
369	5	Lente	32	10	12	32	34	21	0	−24	−9
373	6	Lente	40	54	36	40	45	33	0	8	3
374	6	Lente	32	70	41	33	73	50	−1	−3	−9
378	6	Rapitard	36	97	53	36	124	56	0	−27	−3
383	6	Rapitard	28	4	10	28	7	11	0	−3	−1
384	6	Lente	51	23	19	40	20	17	11	3	2
409	6	Rapitard	28	69	45	28	88	50	0	−19	−5
411	6	Lente	26	41	25	27	48	42	−1	−7	−17
414	6	Lente	35	41	45	32	12	43	3	29	2
415	6	Lente	15	18	31	15	45	30	0	−27	1
416	6	Lente	28	40	25	27	90	33	1	−50	−8
417	6	Lente	37	31	27	43	9	21	−6	22	6
418	6	Lente	28	69	39	25	68	44	3	1	−5
421	6	Lente	28	52	28	28	90	38	0	−38	−10
422	6	Lente	28	79	37	26	42	30	2	37	7
423	6	Lente	44	71	43	46	35	27	−2	36	16
428	6	Lente	35	73	41	33	87	53	2	−14	−12
429	6	Rapitard	35	23	30	32	28	23	3	−5	7
430	6	Lente	20	10	15	20	11	14	0	1	1
431	6	Lente	60	103	51	56	103	50	4	0	1
432	6	Lente	36	20	33	36	25	25	0	−5	8
433	6	Rapitard	45	52	32	44	62	41	1	−10	−9
434	6	Lente	40	49	44	41	40	27	1	9	17
435	6	Lente	44	58	42	36	93	54	8	−35	−12
436	6	Rapitard	32	26	23	31	19	20	1	7	3
437	6	Rapitard	36	18	24	32	34	26	4	−16	−2
438	6	Lente	43	61	37	40	82	40	3	−21	−3
439	6	Lente	36	10	22	36	25	26	0	−15	−4
440	6	Lente	48	30	55	40	111	68	8	−81	−13
444	6	Rapitard	30	21	33	31	68	47	−1	47	−14
445	6	Rapitard	36	84	43	36	69	45	0	15	−2
446	6	Rapitard	44	93	52	44	68	48	0	25	4
447	6	Lente	25	27	34	30	26	28	5	1	6
448	6	Rapitard	24	10	28	24	36	37	0	−26	−9
449	6	Rapitard	33	4	26	32	43	42	1	39	−16
451	6	Rapitard	32	48	31	32	49	31	0	1	0
452	6	Rapitard	32	118	53	34	79	46	−2	39	7

Table VI (Cont)

n = 50			1 × Rap tard			1 × Lente					
Case	No of days per period	Insulin first given	Dose	$M^{BS}9$	M	Dose	$M^{BS}9$	M	Δ Dose	Δ $M^{BS}9$	Δ M
453	6	Lente	41	24	27	44	44	28	3	−20	−1
454	6	Rap tard	51	94	39	48	57	35	3	37	4
455	6	Rap tard	38	38	41	34	57	42	4	−14	−1
456	6	Rap tard	45	29	27	43	46	39	2	−17	−12
457	6	Rap tard	55	28	30	53	75	40	2	−47	−10
458	6	Rap tard	34	6	16	32	18	19	2	−12	−3
459	6	Lente	31	39	34	34	14	28	3	25	6
461	6	Rap tard	40	34	20	40	61	31	0	27	−11
463	6	Rap tard	34	17	26	30	42	40	4	−25	−14
Average			36 6	42 9	32 7	35 5	51 7	35 5	1 1	−8 9	−2 7
S E									0 4	3 6	1 1
P %									2	2	2

f 31 0 62% (46 —75%) (P 5%)

wh h is better for the group as a whole is 100 %. The table also states the stat st cal confidence l mits
(P = 5 %) of f (72 % — 100 %)

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Out of the 114 d abet cs 95 were used for one comparison wh le 19 were used n two or more of the total of 136 comparisons

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One injection therapy

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The mean M value as well as the $M^{BS}9$ value were lower on Rapitard which gave better control (lower M value) in about two-th rds of the 50 cases In order to illustrate the compari sons the blood sugar curves for the first

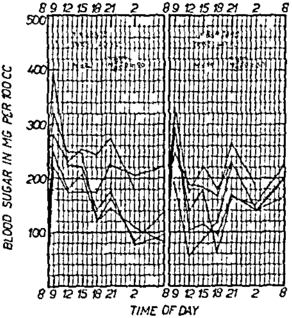


Fig 1 A comparison in one case between the actions of Rapitard and Lente given once daily (case No 332) Each period had 5 days of observation

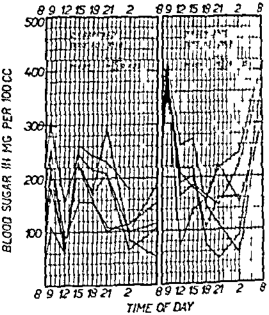


Fig 2 Case 377 showing the most pronounced difference in response (M value and $M^{BS}9$-value) between Rapitard and Semilente when used in two daily injections

patient in the table (no 332) are shown in fig 1

Two injection therapy

In cases treated by two daily injections better control is obtained if the insulin crystal suspension has an appreciable content of dissolved insulin In a group of 13 patients the 25 % suspension, Rapitard, was found to give significantly ($P = 3$ %) lower $M^{BS}9$ values than the 40 %-suspension while the M-values were not significantly lower ($P = 20$ %) Since the 25 % suspension is preferred for one-injection therapy and may be mixed, if desired with Actrapid this suspension Rapitard was selected for the further comparative studies Table III showed how two injections of Rapitard gave considerably better results than two injections of S I Insulin Semilente is used to some extent for treatment by two daily injections and is therefore represented in table VII

Even in a group of only 11 patients ΔM_{gr} is clearly in favour of Rapitard ($P = 1$ %) as illustrated in fig 2 which presents the most striking case no 377

It is evident from table VII and fig 2 that compared with Semilente, Rapitard had a stronger effect upon the 9 a m blood sugar Compared with one injection of Lente one injection of Rapitard was better (table VI) and two injections still better (table VIII series 4) Particular interest attaches to the comparison (table VIII series 5) between two injections of Rapitard and the two injection therapy which has so far been so widely used at the Hvidore Hospital viz a mixture of Lente and Semilente in the morning and Lente in the evening (L + S/L) In this series

TABLE VII Comparison of Rap tard insul n and Semilente both adm n stered in two da ly inject ons

n 11			2 × Rap tard			2 × Semilente					
Case	No of days per per od	Insulin first given	Dose	$M^{BS}9$	M	Dose	$M^{BS}9$	M	Δ Dose	Δ $M^{BS}9$	Δ M
355	6	Sem lente	27/9	5	24	26/15	55	33	− 5	− 50	− 9
357	6	Rap tard	33/7	26	27	29/ 9	138	59	2	−112	−32
358	6	Rap tard	28/8	50	26	25/12	79	34	− 1	− 29	− 8
359	6	Sem lente	31/9	21	24	25/12	34	22	3	− 13	2
365	6	Sem lente	18/9	40	35	20/12	127	54	− 4	− 87	−19
372	6	Semilente	28/8	39	34	24/ 8	104	45	4	− 65	−11
377	6	Rap tard	35/8	23	27	24/ 8	135	61	7	−112	−34
380	6	Rap tard	28/8	38	20	28/ 8	54	24	0	− 16	− 4
381	6	Rap tard	33/8	2	13	32/ 8	27	20	1	− 25	− 7
382	6	Semilente	19/4	17	31	35/12	51	34	−24	− 34	− 3
386	6	Rap tard	25/8	13	46	24/11	86	46	− 2	− 73	0
Average			35 5	24 9	27 9	37 4	80 9	39 3	−1 82	−56 0	−11 4
S E									2 5	11 0	3 6
P									60	0 1	1

f 9 11 82 (48%—98 %) P ≈ 5 %)

TABLE VIII The evalua on of Rap tard n 5 series of comparisons

Series No	Compa ison	No of cases	Dose Δ	Dose P %	$M^{BS}9$ Δ	$M^{BS}9$ P %	$\bar{M}$ Δ	$\bar{M}$ P %	Av dose on Rap
1	1 × Rap 1 × Lente	50	1	2	− 9	2	− 3	2	37
2	2 × Rap —2 × S 1	11	22	<0 1	−81	<0 1	−21	<0 1	43
3	2 × Rap —2 × Semilente	11	2	60	−56	<0 1	11	1	36
4	2 × Rap 1 Len e	38	1	10	−32	<0 1	− 9	<0 1	42
5	2 Rap —Len e × Se L	26	1	0	−22	<0 1	− 4	2	39

the average dose of the three fractions was 20 8 /₂ and 10 /₃ i u respec t vely

Discussion

The investigat ons were based on a clin ical model wh ch is not a random sample of the insul n treated diabetic population Nevertheless the consecu tively hospitalized pat ents presumably make up a sufficiently representative material since there seems to be no relationsh p between age sex dosage and labil ty on the one hand and the sign of ΔM on the other (i e which of the two compared treatments is bet

ter) On the other hand there is a relationship between the patients' lability and the size of the numerical ΔM-value, as patients who are labile and difficult to control also change more when shifted to another insulin *Exclusion of the* stable cases makes the material on the whole more sensitive The influence exerted by the conditions of living on ΔM (the difference between the efficacy of the treatments) could not be determined These aspects might perhaps be elucidated by an investigation of the series on an outpatient basis, but this could not be done within the scope of the present study

The M-formula was discussed in a previous paper (17) In the present paper it is used as a criterion for deciding which insulin treatments are better than others The clinical importance of ΔM is a question apart As a main rule an effort is made to improve the patients' "control" as far as is permitted by practical economic and psychological considerations

In all 5 series (table VIII) in one-injection as well as two injection therapy Rapitard provided a better control of the hyperglycaemic peak value at 9 a m The explanation is that Rapitard is a *combination of quick-acting S I (25 %)* and intermediate insulin (75 %) Neither S I alone (series 2) nor intermediate insulin alone (series 1 3, 4, and 5) was as effective in counteracting the postprandial increase in the morning blood sugar In one injection as well as two injection therapy Rapitard gave a significantly better result than the Lente preparations (series 1, 2, 3, 4, and 5 in table VI) This is not saying that the Lente-treated patients should be transferred to Rapitard, as better control on Rapitard was not the invariable rule On the other hand, the results indicate that Insulin Rapitard would be a valuable *supplement in one injection as* well as two injection therapy So far, the clinical experience of Rapitard has confirmed the assumption regarding its clinical applicability (2, 6, 19, 20)

Insulin Actrapid is employed occasionally in combination with Rapitard and moreover as S I Owing to its neutral reaction it is absorbed more rapidly than the usual acid S I

Summary

Rapitard is a new insulin preparation containing 25 % dissolved quick acting insulin (Actrapid) and 75 % suspended ox insulin crystals of intermediate action (i e approximately like Lente or NPH) Actrapid is a new quick-acting S I made of pure porcine insulin It has neutral reaction (pH 7) and is absorbed more quickly than the usual acid S I

The clinical study comprised 114 consecutively *hospitalized, insulin treated* and labile diabetic patients of both sexes ranging in age from 7 to 67 Insulin *Rapitard was compared in one injec*tion therapy with Lente and in two injection therapy with S I Semilente, and a combination of Lente Semilente The clinical results were evaluated by the use of the M-formula described in a previous paper In all five series Rapitard gave *better control of* the blood sugar, both at the time of its peak value at 9 a m and throughout the 24 hours

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ter). On the other hand there is a relationship between the patients' lability and the size of the numerical ΔM-value, as patients who are labile and difficult to control also change more when shifted *to another insulin*. Exclusion of the stable cases makes the material on the whole more sensitive. The influence exerted by the conditions of living on ΔM (the difference between the efficacy of the treatments) could not be determined. These aspects might perhaps be elucidated by an investigation of the series on an outpatient basis, but this could not be done within the scope of the present study.

The M-formula was discussed in a previous paper (17). In the present paper *it is used as a criterion for deciding* which insulin treatments are better than others. The clinical *importance of* ΔM is a question apart. As a main rule an effort is made to improve the patients' "control" as far as is permitted by practical economic and psychological considerations.

In all 5 series (table VIII) in one-injection as *well* as two-injection *therapy* Rapitard provided a better control of the hyperglycaemic peak value at 9 a.m. The explanation is that Rapitard is a *combination* of quick-acting S.I. (25 %) and intermediate insulin (75 %). Neither S.I. alone (series 2) nor intermediate insulin alone (series 1, 3, 4, and 5) was as effective in counteracting the *postprandial increase in the morning* blood sugar. In one-injection as well as two-injection therapy Rapitard gave a significantly better result than the Lente preparations (series 1, 2, 3, 4, and 5 in table VI). This is not saying that the Lente-treated patients should be transferred to Rapitard, as better control on Rapitard was not the invariable rule. On the other hand, the results indicate that Insulin Rapitard would be a *valuable supplement* in one *injection as* well as two-injection therapy. So far, the clinical experience of Rapitard has confirmed the assumption regarding its clinical applicability (2, 6, 19, 20).

Insulin Actrapid is employed occasionally in combination with Rapitard and moreover as S.I. Owing to its neutral reaction it is absorbed more rapidly than the usual acid S.I.

Summary

Rapitard is a new insulin preparation containing 25 % dissolved quick-acting *insulin (Actrapid)* and 75 % suspended ox insulin crystals of intermediate action (i.e. approximately like Lente or NPH). Actrapid is a new quick-acting S.I. made of pure porcine insulin. It has neutral reaction (pH 7) and is absorbed more quickly than the usual acid S.I.

The clinical study comprised 114 consecutively hospitalized, insulin-treated and labile diabetic patients of both sexes ranging in age from 7 to 67. Insulin Rapitard was compared in one-injection therapy with Lente and in two-injection therapy with S.I., Semilente and a combination of Lente-Semilente. The clinical results were evaluated by *the use of the M formula* described in a previous paper. In all five series Rapitard gave better control of the blood sugar both at the time of its peak value at 9 a.m. and throughout the 24 hours

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Serum Lactic Dehydrogenase Activity and Isoenzyme Patterns in Some Haematological Diseases

By

JOUKO A PALOHEIMO and EERO IKKALA

The determination of serum lactic dehydrogenase (LDH) activity is widely used in clinical diagnosis (2, 26) The value of this test in differential diagnosis is not great because activity changes can be seen in many diseases, including myocardial infarction pulmonary embolism various blood disorders malignancies and hepatic diseases Fractionation of LDH activity of serum into five isoenzymes can be of more help clinically because the isoenzyme pattern of serum may resemble that of the diseased organ (1 10 11 15 16, 21—23 29) In this work the fastest isoenzyme electrophoretically is called LDH_1 and the slowest LDH_5, the isoenzymes with intermediate electrophoretic mobility are LDH_2, LDH_3 and LDH_4

An elevated total serum LDH activity is often observed in patients with various blood disorders In megaloblastic and haemolytic anaemias it is almost always elevated (4, 5, 9, 27) and this rise is due to an increase in fractions LDH_1 and LDH_2 (3, 8) According to a review of literature (2) about 60 per cent of patients with leukaemias have a high serum LDH level, which is caused primarily by elevation of fractions LDH_2 and LDH_3 There have also been reports of normal or low normal total serum LDH activities in sideropenic anaemia (18) and normal or elevated activities in aplastic anaemia (2 17) myelofibrosis and polycythaemia vera (13 14), but no attention has been paid to the isoenzyme patterns either in these diseases or in multiple myeloma

In the present study, total LDH activities and LDH isoenzyme patterns were measured in a group of 57 patients with different blood disorders, in order to estimate the value of such determinations in haematological diagnosis

Material

The present series consists of 16 young healthy males and females and of 57 hospital patients with various haematological disorders

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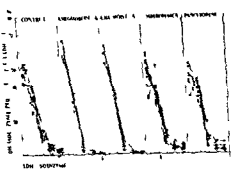


Fig 2

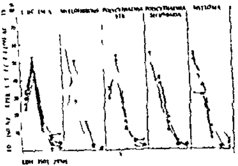


Fig 3

Figs 2 and 3 Serum LDH isoenzymes as per cent of total LDH activity in control and disease groups
● – cases with total LDH activity < 500 U
○ = cases with total LDH activity > 500 U

LDH_1 When the total LDH activity was low, LDH_1 contained up to 55 per cent of the total activity

In all patients with megaloblastic and in three of five with haemolytic anaemia the total LDH activity was elevated This rise was due to an increase in fractions LDH_1 and LDH_2 These fractions were also elevated in the 2 patients with haemolytic anaemia who had a normal total LDH activity

In pancytopenia the total LDH activity varied from low to slightly elevated The isoenzyme pattern was normal in all One of the patients with an elevated total LDH activity (550 U) also showed a slight haemolysis and over 50 per cent of all enzyme activity was in LDH_1 LDH_2 was higher than fraction LDH_1 in the patient with pure agranulocytosis

Total LDH activity was normal or low normal in all cases of iron deficiency anaemia and the isoenzyme pattern was also normal In three patients LDH_2 was slightly higher than LDH_1 but these patients showed no obvious differences clinically from the others (One patient had iron deficiency due to bleeding from carcinoma of the stomach and had an increase in isoenzyme LDH to 9 1 per cent of the total activity This increase was thought to be caused by the malignancy, and the patient was excluded)

All patients with leukaemia had slightly or moderately elevated total LDH levels The isoenzyme patterns in all showed increases in fractions LDH_2 and LDH_3 No clear differences between different types of leukaemia could be found In myelofibrosis and polycythaemia vera the total LDH activities were within the normal range, except for one patient with polycythaemia vera who had leukaemic features (leucocyte count 50,000/mm³ and a marked shift to left in the differential) and a total LDH activity of 560 U The isoenzyme patterns in all of these patients corresponded to that seen in leukaemia, i e the greatest activity was in LDH_2 fraction Three of five patients with secondary polycythaemia had normal total LDH activities and isoenzyme patterns The other two had

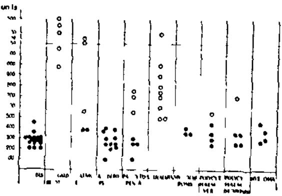


Fig. 1. Serum LDH activity of controls and in disease groups.
● = total activity < 500 U
○ = total activity > 500 U

Five patients with megaloblastic anaemia (Addisonian pernicious anaemia (4) and broad tapeworm anaemia (1)),

Five patients with haemolytic anaemia (congenital spherocytosis (2), autoimmune haemolytic anaemia (1) and acquired Coombs negative haemolytic anaemia of unknown cause (2)),

Eight patients with pancytopenia, which in 3 patients was obviously due to chemicals and in one to roentgen therapy. In three no definite cause could be found. One patient with pure agranulocytosis is included to this group,

Thirteen patients with iron-deficiency anaemia. Haemoglobin level ranged from 5,4 to 11 g/100 ml and all patients had mean corpuscular haemoglobin concentrations below 28, serum iron levels below 30 γ g/100 ml and total iron binding capacities over 350 γ g/100 ml,

Two patients with acute leukaemia (myeloblastic (1) and myelomonocytic (1)).

Seven patients with chronic leukaemia (granulocytic (4) and lymphatic (3)),

Three patients with primary myelofibrosis in all of whom diagnosis was confirmed by bone-marrow biopsy.

Four patients with polycythaemia vera and 5 patients with secondary polycythaemia due to congenital heart disease.

Five patients with multiple myeloma, all having numerous myeloma cells in the bone marrow and tall, narrow paraprotein peaks of β- or γ mobility in paper electrophoresis. In addition, 4 patients showed typical osseous changes.

Methods

Enzyme activities were determined using fasting blood serum. Samples with haemolysis were discarded. All sera were kept at 4 to 6° C and studied within 6 hours after they were obtained. The total activity of LDH was determined as Wróblewski units (28) using laboratory kits manufactured by Biochimica Boehringer & Soehne, Germany. In our laboratory normal values are less than 500 units. LDH isoenzymes were studied using the enzyme-electrophoresis method of van der Helm (7). Agar gel electrophoresis of 10 μ l of serum was run on microscope slides (24) in an immunoelectrophoresis apparatus (Agaphor) at 40—50 volts and 100 mA for 45 minutes. Difco Special Agar Noble (0.8 %) was used. Room temperature was held at 4 to 6° C to avoid heat inactivation of the slow moving isoenzymes. Enzyme activities were visualized as described by van der Helm et al. (8) by incubating the slides for 2 hours at 37° in the dark in a medium containing diphosphopyridine nucleotide, sodium lactate, nitroblue tetrazolium and phenazine methosulphate in a phosphate buffer solution. Thereafter the amount of activity in each fraction was determined using the photometric curve for planimetration. The amount of each isoenzyme is expressed as a percent of the total enzyme activity.

Results

The total serum LDH activities for each disease group and the control group are given in fig. 1. The percentage of each LDH isoenzyme in each case is shown also according to disease groups in figs. 2 and 3.

The isoenzyme patterns of normal controls showed the predominance of

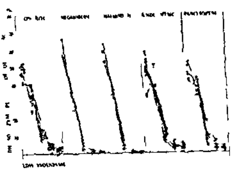


Fig 2

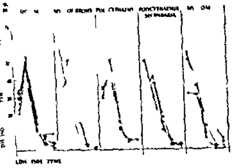


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Total LDH activity was normal or low normal in all cases of *iron-deficiency* anaemia and the isoenzyme pattern was also normal In three patients LDH_2 was slightly higher than LDH but these patients showed no obvious differences clinically from the others (One patient had iron deficiency due to bleeding from carcinoma of the stomach and had an increase in isoenzyme LDH_4 to 9 1 per cent of the total activity This increase was thought to be caused by the malignancy and the patient was excluded)

All patients with leukaemia had slightly or moderately elevated total LDH levels The isoenzyme patterns in all showed increases in fractions LDH_2 and LDH_3 No clear differences between different types of leukaemia could be found In myelofibrosis and polycythaemia vera the total LDH activities were within the normal range except for one patient with polycythaemia vera who had leukaemic features (leucocyte count 50 000/mm³ and a marked shift to left in the differential) and a total LDH activity of 560 U The isoenzyme patterns in all of these patients corresponded to that seen in leukaemia i e the greatest activity was in LDH_2 fraction Three of five patients with secondary polycythaemia had normal total LDH activities and isoenzyme patterns The other two had

isoenzyme patterns corresponding to that seen in polycythaemia vera, and one of these also had an elevated total LDH-activity

Four of the patients with multiple myeloma had normal total LDH activities and normal isoenzyme patterns The fifth patient had elevated LDH_3- and LDH_4-levels

Discussion

In the sera of patients with megaloblastic and haemolytic anaemias, high LDH activities due to elevation of fractions LDH_1 and LDH_2 were observed These are the two fractions that predominate in red blood cells (11, 20, 25) In addition, the total LDH activity of megaloblastic marrow is known to be higher than normal (6) and to have an isoenzyme pattern with a higher percentage of LDH_1 than normal marrow (31) Thus it seems plausible that the rise of LDH_1 and LDH_2 in these types of anaemia is partly due to haemolysis and partly to a release of enzyme activity from the marrow

In the patients with pancytopenia no typical findings were seen either in the total activities or in the isoenzyme patterns This may be due to the different aetiological factors involved and the different disease stages seen Elevated total LDH values were observed in three of seven patients In one of these the rise could have been caused by accompanying slight haemolysis, and in the two other patients no clear explanation was found, but elevated total LDH values have been earlier reported in terminal aplastic anaemia (2)

In sideropenic anaemia the total LDH activities were low normal or normal, thus confirming earlier observations (18) The isoenzyme pattern was also normal

It would seem that the determination of isoenzyme levels of serum LDH does not have great value in differential diagnosis between anaemias, except for megaloblastic anaemias with very high LDH activities But these determinations may be valuable in differential diagnosis of icterus (30), because the isoenzyme patterns in haemolytic disorders differ significantly from those in hepatic disease, in which LDH_5 is elevated

Both normal and leukaemia leucocytes and bone marrow have LDH activity with predominance of fractions LDH_2 and LDH_3 (12, 19, 20, 31) Thus the elevated enzyme levels and "leukaemic" (LDH_2—LDH_3) isoenzyme patterns seen in patients with leukaemia may partly reflect the increased number of leucocytes present and the greater activity of the marrow found in these patients The similar patterns seen in patients with lymphatic and myeloid leukaemias are explained by the failure to detect different isoenzyme patterns in marrow extracts from such patients (31) In other myeloproliferative diseases, in myelofibrosis and in polycythaemia vera, the total LDH activities were normal in most cases, but the isoenzyme pattern corresponded to that of leukaemia LDH_4 pattern was observed also in two of five patients with secondary polycythaemia It is of interest to note that in malignant diseases as carcinomatosis, the "leukaemic" pattern of LDH isoenzymes is frequently seen, but in these cases fractions LDH_3 and LDH_4 and

especially LDH_5 may also be elevated In our series one patient with sideropenic anaemia had carcinoma and LDH_5 fraction was elevated

The present observations that both total LDH activity and isoenzyme patterns in sera from myeloma patients were normal may suggest that the metabolic pathways of myeloma cells differ from those of leukaemic cells The isoenzyme pattern of myeloma cells is unknown

The determination of serum LDH-isoenzymes has no great value in differential diagnosis of myeloproliferative diseases, because all of them show the same LDH_2 pattern Differential diagnosis between polycythaemia vera and secundaria is likewise not possible with LDH isoenzymes, but the determination of them may give some help

Summary

Total serum LDH activities and LDH isoenzyme patterns were measured to determine their value in haematological diagnosis in a series of 57 patients with different blood disorders The method used for isoenzyme studies was agar gel electrophoresis

Increased total LDH activity with an isoenzyme pattern showing high LDH_1— and LDH_2 — fractions was observed in cases of megaloblastic anaemia and haemolytic anaemia In pancytopenia no typical findings were seen In sideropenic anaemia total LDH activities and isoenzyme patterns were normal In leukaemic serum the total activity was elevated and isoenzyme pattern showed greatest activity in LDH_2 LDH isoenzyme patterns in myelofibrosis and polycythaemia vera were also of the LDH_2 type, but in polycythaemia secundaria LDH_1 patterns were also seen In the serum of myeloma patients the total LDH activity and the isoenzyme patterns were normal

It is concluded, that LDH activity determination is not of great value in haematological diagnosis Very high activities may be pathognomonic for megaloblastic anaemia, but in differential diagnosis among other anaemias and among myeloproliferative diseases the determination of total activity and isoenzyme patterns of LDH gives little information

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From the Third Department of Medicine University of Helsinki Helsinki Finland

Effect of Equi-pressor Doses of Synthetic α- and β-Angiotensin II on Urinary Volume and Urinary Electrolytes in Normotensive Subjects

By

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β Asp[1] Val[5] Angiotensin II differs from α Asp[1] Val[5] Angiotensin II in molecular structure solely in the respect that the asparaginic acid is bonded to arginine in the β instead of the α position The principal difference in the pharmacological properties of the two angiotensins has been found in their vasopressor effect This effect of β angiotensin II clearly exceeds that of α angiotensin II (3)

In the present study the effect of β angiotensin II and α angiotensin II on diuresis and on the urinary excretion of sodium and potassium was followed under conditions in which the vasopressor response to the angiotensins was equally strong

Material and methods

The series consisted of 10 normotensive women 21 to 59 years of age No cardiovascular or renal disease had been diagnosed previously or at the time of the test The subjects had fasted for 10 hours and 1 1/2 hours before the test was begun they drank about 1 litre of water The bladder was emptied by a catheter which remained in the bladder throughout the test The experiment lasted 150 min divided into five successive periods of 30 min each During each period the amount of urine excreted into the bladder was measured and the urinary sodium and potassium were determined by flame photometry The mean urinary volumes and mean urinary sodium and potassium values of the first two periods were used as initial values Immediately after the second period β angiotensin II was injected intramuscularly in an amount sufficient to raise the systolic and diastolic blood pressures 20—30 mm Hg above the initial levels The dose of β angiotensin II required to attain this vasopressor effect had been determined on the preceding day The product used was β Asp[1] Val[5] Hypertensin II (Ciba 33902 Ba) developed by the Ciba Laboratories Blood pressure measurements were made at 5 min intervals until the pressor reverted to the initial level The test was repeated on the same subjects on the following day with the difference that during period III α Asp[1] amide Angiotensin II (Hypertensin® Ciba) dissolved in a 5 % glucose solution was given intravenously by drip infusion The rate of drip was adjusted

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TABLE I Percentage of change in urinary volume (U_{Vol}), urinary sodium (U_{Na}) and urinary potassi
sin II infusion Each period lasted 30 min The means of period I and II were used as con
on the following day α angiotensin II was given as an intravenous drip infusion during pe

Subject	Age	Derivative of angiotensin II and dosage	Period III			Period IV			Period V		
			U_{Vol}	U_{Na}	U_K	U_{Vol}	U_{Na}	U_K	U_{Vol}	U_{Na}	U_K
F J	59	β 100 μg	−30	−2	+48	−51	−26	−3	−78	−79	−57
		α 0 93 μg/min	−33	−3	−66	−53	−47	−76	−80	−72	−87
A L	47	β 125 μg	−34	−2	+19	−60	−15	−13	−36	+105	+59
		α 1 1 μg/min	−20	+29	−12	−36	+56	−26	+10	+324	+7
M A	56	β 125 μg	−84	−70	−69	−95	−90	−89	−48	+82	+80
		α 2 1 μg/min	−95	−87	−87	−95	−81	−79	−79	−8	−18
E A	28	β 150 μg	−71	−45	−46	−97	−94	−81	−90	−54	−48
		α 2 3 μg/min	−66	−29	−35	−87	−70	−5	−89	−46	−46
M V	21	β 150 μg	−55	−20	−6	−91	−34	−50	−86	+27	−29
		α 2 6 μg/min	−39	−29	−34	−84	−63	−38	−87	+7	+2[illegible]
K J	35	β 150 μg	−73	−30	−33	−92	−69	−62	−87	+22	+2
		α 1 9 μg/min	−88	−76	−80	−94	−74	−74	−89	+90	+39
A B	33	β 150 μg	−39	−36	−35	−70	−46	−45	−94	−67	−80
		α 1 0 μg/min	+13	+36	+5	−85	−12	−59	−70	−81	−66
A R	49	β 150 μg	−32	−58	−53	−44	−71	−60	−12	−54	−47
		α 1 2 μg/min	−41	−49	−61	−77	−29	−48	−33	−5	−55
L R	38	β 150 μg	−81	−76	−71	−91	−71	−35	−90	−55	−44
		α 1 2 μg/min	−68	−68	−68	−83	−73	−69	−59	−2	−29
I F	53	β 150 μg	−96	−96	−94	−71	−67	−55	−36	−25	−44
		α 1 0 μg/min	−84	−84	−86	−1	−3	−55	−41	−36	−81

so that the systolic and diastolic blood pressures during period III followed exactly the same course as the changes in blood pressure produced on the preceding day by β angiotensin II

Results

Vasopressor effect A blood-pressure rise of 20—30 mm Hg was produced in seven normotensive subjects by an intramuscular injection of 150 μg of β angiotensin II, in two subjects by 125 μg, and in one by 100 μg (table I) With one exception the vasopressor effect was already evident within 5 min and lasted on the average 15 min In two cases only (I J A R) did the blood pressure remain elevated for 30 min after the β angiotensin II injection The test system did not allow a comparison between the vasopressor effects of the two angiotensins The rate of infusion of α angiotensin was entirely dependent on the vasopressor response to β angiotensin on the preceding day and it was necessary to lower the rate of drip of

um (U_K) in normotensive subjects influenced by β angiotensin II intramuscularly and by α angioten trols. β angiotensin II was given intramusculary in the beginning of period III on the first day and riod III at a rate causing the same vasopressor response

Blood pressure (mm Hg) during period III and 40 min after i m injection of β ang II							
0 min	5 min	10 min	15 min	20 min	25 min	30 min	40 min
165/90	185/105	190/115	190/110	180/105	180/105	180/105	170/90
135/90	170/120	155/115	150/110	140/100	140/100	135/90	—
140/80	145/90	165/105	160/95	155/90	145/85	140/80	—
120/90	145/105	135/100	130/100	125/90	120/85	120/85	—
125/95	145/110	140/105	135/100	130/95	130/95	130/90	—
120/90	130/110	140/115	135/110	125/105	125/100	120/90	—
130/80	150/100	160/105	150/100	145/95	140/90	135/85	—
130/80	130/80	160/100	170/105	165/100	160/100	150/90	135/85
110/70	155/90	170/105	155/95	145/85	120/80	125/80	115/70
110/75	140/95	165/100	155/95	150/85	145/80	130/75	120/70

α angiotensin to conform with the weakening of the effect of β angiotensin towards the end of period III The statement can only be made that the individual α angiotensin doses needed for the attainment of the same level of vasopressor response varied over a considerably wider range than the β angiotensin dosages

Effect on diuresis and urinary electrolytes During period III when the vasopressor effect of β angiotensin was strongest the urinary volume decreased in all cases The urinary excretion of sodium diminished in eight cases and was practically unchanged in two cases The excretion of potassium during period III under the influence of β angiotensin followed a trend similar to that of sodium The only difference occurred in the two cases in which the urinary sodium during period III was unchanged, while the urinary potassium increased under the influence of β angiotensin II

Although the vasopressor response to β angiotensin II was gone in most cases

TABLE I. Percentage of change in urinary volume (U_{Vol}), urinary sodium (U_{Na}) and urinary potassi- sin II infusion. Each period lasted 30 min. The means of period I and II were used as con- on the following day α angiotensin II was given as an intravenous drip infusion during pe-

Subject	Age	Derivative of angiotensin II and dosage	Period III			Period IV			Period V		
			U_{Vol}	U_{Na}	U_K	U_{Vol}	U_{Na}	U_K	U_{Vol}	U_{Na}	U_K
T. J.	59	β 100 μg	−30	−2	+48	−51	−26	−3	−78	−79	−57
		α 0.93 μg/min	−33	−3	−66	−53	−47	−76	−80	−72	−87
A. L.	47	β 125 μg	−34	−2	+19	−60	−15	−13	−36	+105	+59
		α 1.1 μg/min	−20	+29	−12	−36	+56	−26	+10	+324	+7
M. A.	56	β 125 μg	−84	−70	−69	−95	−90	−89	−48	+82	+80
		α 2.1 μg/min	−95	−87	−87	−95	−81	−78	−79	−8	−18
E. Å.	28	β 150 μg	−71	−45	−46	−97	−94	−81	−90	−54	−48
		α 2.3 μg/min	−66	−29	−35	−87	−70	−5	−89	−46	−46
M. V.	21	β 150 μg	−55	−20	−6	−91	−34	−50	−86	+27	−29
		α 2.6 μg/min	−39	−29	−34	−84	−63	−38	−87	+7	+27
K. J.	35	β 150 μg	−73	−30	−33	−92	−69	−62	−87	+22	+2
		α 1.9 μg/min	−88	−76	−80	−94	−74	−74	−89	+90	+39
A. B.	33	β 150 μg	−39	−36	−35	−70	−46	−45	−94	−67	−80
		α 1.0 μg/min	+13	+36	+5	−85	−12	−59	−70	−81	−66
A. R.	49	β 150 μg	−32	−58	−53	−44	−71	−60	−12	−54	−47
		α 1.2 μg/min	−41	−49	−61	−77	−29	−48	−33	−5	−55
L. R.	38	β 150 μg	−81	−76	−71	−91	−71	−35	−90	−55	−44
		α 1.2 μg/min	−68	−68	−68	−83	−73	−68	−59	−2	−29
I. F.	53	β 150 μg	−96	−96	−94	−71	−67	−55	−36	−25	−44
		α 1.0 μg/min	−84	−84	−86	−1	−3	−55	−41	−36	−81

so that the systolic and diastolic blood pressures during period III followed exactly the same course as the changes in blood pressure produced on the preceding day by β angiotensin II.

Results

Vasopressor effect. A blood pressure rise of 20—30 mm Hg was produced in seven normotensive subjects by an intramuscular injection of 150 μg of β angiotensin II, in two subjects by 125 μg, and in one by 100 μg (table I). With one exception the vasopressor effect was already evident within 5 min and lasted on the average 15 min. In two cases only (I. J., A. R.) did the blood pressure remain elevated for 30 min after the β angiotensin II injection. The test system did not allow a comparison between the vasopressor effects of the two angiotensins. The rate of infusion of α angiotensin was entirely dependent on the vasopressor response to β angiotensin on the preceding day, and it was necessary to lower the rate of drip of

and sodium excretion seen in both tests may be the vasoconstriction of the renal afferent arteries and reduction of the glomerular filtration rate produced by both angiotensins Since, however, the changes in the urinary volumes and sodium excretion continued for a considerably longer time than the general vasopressor effect of the angiotensins, it does not appear probable that the effect of angiotensin on the tubular passage of electrolytes is solely dependent on the vasoconstriction To maintain a continued marked reduction of the glomerular filtration rate the angiotensin should cause vasoconstriction of the renal afferent arteries selectively and for a considerably longer time than in other arteries It has been shown in man that after the infusion of synthetic angiotensin the glomerular filtration soon reverts to the initial level or above it (2) Attempts have therefore been made to clarify whether or not angiotensin also has a direct effect, independent of vasoconstriction, on the tubular passage of sodium From animal experiments it has been stated that α angiotensin inhibits the reabsorption of sodium in the distal tubules (7, 9) In tests with normotensive human subjects, however, α angiotensin has been observed to reduce the urinary excretion of sodium (1, 2 5, 6 8) The use of non physiologically high intrarenal angiotensin concentrations in animal experiments might be one reason for the divergence of the results Hence the results obtained in animal experiments probably are not directly comparable with the possible physiological function of angiotensin in the mechanism of electrolyte regulation in the human kidney Possibly the intrarenal concentration of angiotensin has an important role in the regulation of electrolyte reabsorption and excretion by the kidneys, since, as shown by experiments using isotopes, angiotensin has a tendency to accumulate in the kidneys (4) Thus changes may still occur in the excretions of the urinary electrolytes after the general vasopressor effect of angiotensin has passed, if the intrarenal concentration of angiotensin exceeds the original physiological level but the total concentration is inadequate for the production of general vasoconstriction

Summary

Ten normotensive women were given equi pressor doses of synthetic β angiotensin II intramuscularly and synthetic α angiotensin II by intravenous drip infusion Observations were made of the diuresis and the urinary excretion of sodium and potassium The effect of the two angiotensins on the urinary volume and electrolyte excretion was similar in trend both during the vasopressor response and also after the vasopressor effect of the angiotensins had passed

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30 min after the intramuscular injection, the change from initial values that took place in the urinary volume and the amount of urinary electrolytes during period *III* continued during period IV or even, in a proportion of the cases, was enhanced In period V, i e from 60 to 90 min after the injection of β angiotensin II, the urinary volume continued to be lower than before the injection in most cases A reversion of the urinary electrolyte excretion towards the initial values began in period V and in some cases exceeded them within this period

A comparison of the effect of the two angiotensins on the diuresis and urinary electrolytes during period III displays an unexpectedly similar effect Only in one case were the urinary volume and *electrolytes decreased during period III* after β-angiotensin II, while during α angiotensin II infusion these values increased over the initial levels However, even in this case (A B) the changes in these values during the following 30 min period had a similar trend in both the β- and the α angiotensin tests Of the two subjects (F J and A L) in whom β angiotensin II hardly affected the sodium excretion during period III, one (F J) showed no change in this excretion in the corresponding period even *during α angiotensin II infusion* In the later periods too the urinary sodium of this subject followed the same trend in both angiotensin tests In the other case (A L) the α angiotensin II infusion increased the urinary sodium value in period III above the initial level There was still a marked dif*ference in the sodium excretion between* the β and α-angiotensin tests in period IV, but during the last period the urinary sodium in this case showed *in both tests* a greater increase over the initial level than in any other case Generally the effects of both angiotensins on the urinary volume and urinary electrolytes during the last period of observation, period V, were similar in trend, with the exception that in the test with α-angiotensin *II* the urinary electrolytes reverted to the initial levels in more cases than after β angiotensin II

Discussion

The vasopressor effect of β angiotensin II was clearly evident in normotensive subjects The blood pressure increased 5 min after an intramuscular injection *and remained elevated for* about 15 min Equally high vasopressor responses were achieved with fairly constant doses, and no marked individual variations were observed Hence, when using β angiotensin II as a vasopressor in the treatment of shock it is well to give a second dose 5 min after the first injection if the *latter has failed to produce the desired* response The dosage necessary in different shock conditions cannot, of course, be estimated from the present study

In the normotensive subjects the β angiotensin II had an antidiuretic antinatriuretic and antikaliuretic action When a general vasopressor effect equal in magnitude to that with *β angiotensin* II was produced by the administration of α angiotensin II, the changes in the urinary volume and the urinary excretion of electrolytes showed a similar trend in both angiotensin tests The possible explanation for decreased diuresis

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Thyro-cytotoxic Factor

Preliminary Report

By

POUL HALBERG

Many features suggest that chronic thyroiditis (Hashimoto's disease) might have an auto immune pathogenesis By now 3 different specific thyroid anti bodies have been demonstrated in sera from patients with this disease, viz a complement fixing antibody against an intracellular, microsomal antigen an antibody against thyroglobulin, and an antibody directed against an antigen in the colloid different from thyroglobu lin The question arises whether any of these serologically demonstrated anti bodies actually have any effect on the thyroid cells or whether they should be considered passive phenomena of no physiological significance

In 1959 Irvine (2) and Pulvertaft et al (5) demonstrated a complement requiring factor in sera from patients with chronic thyroiditis which was specifically toxic to thyroid cell cul tures Later work (1 3, 4) suggested that this cytotoxic factor might be identical with the complement fixing antibody In a previous paper (1) an experiment was reported in which the sensitivity of cell cultures from 42 toxic and atoxic goitres was compared with the complement fixing antigenicity of extracts of the corresponding thyroids using the same standard serum through out the whole experiment There was a suggestion of a correlation, but it was not statistically significant This experi ment has been repeated on 45 new goitres, with two changes in the pro cedure First the cell concentration has been standardized because the titre of the cytotoxic factor tends to go down if the cell concentration is particularly high Secondly the complement fixing antigenicity of the extracts was deter mined by block titration instead of by titration of the extracts at one concen tration only Under these conditions a statistically significant correlation was found between the sensitivity of the cells and the complement fixing antige nicity of the corresponding extracts

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Eosinophilic Leukemia and Disseminated Eosinophilic Collagen Disease — a Disease Entity?

By

Bengt Odeberg

There has always been much scepticism as to the existence of eosinophilic leukemia as a distinct disease entity which it is possible to separate from disseminated eosinophilic collagen disease on one hand and from ordinary myelogenous leukemia with eosinophilia on the other hand In this paper the problem will be discussed in connection with two case reports and a review of recent literature

Case reports

Case 1 A 41 year-old previously healthy man was hospitalized on September 18 1963 with a history of eight days of weakness fever sweats and pains initially located to the right chest but later to the whole body Examination revealed an acutely ill patient who complained of generalized pain there was a questionable enlargement of the inguinal lymph nodes and the temperature was 39 C but otherwise the physical findings were normal

The initial white blood cell count was 11 500 mm³ with 2 % myelocytes 3 5 % metamyelocytes and 19 % eosinophils The hemoglobin concentration was 8 9 g/100 ml Platelets 30 000/mm³ A bone marrow examination (A Hollender M D Centr Chem Lab Sahlgrenska sjukhuset Gothenburg) showed 30 % undifferentiated blast cells and 40 % eosinophils most of which were eosinophilic myelocytes there were no intermediate forms the mature eosinophils showed degenerative characteristics in the form of hypersegmentation and vacuolation of the plasma and the nucleus numerous Charcot Leyden crystals were seen The serum vitamin B_{12} value was 1 100 pg/ml (normal range 175—960 pg/ml) and the haptoglobin level was 250 mg/100 ml There was a slight albuminuria which gradually increased to a maximum of 3 5 ‰, but the urinary sediment as well as the serum creatinine level were normal There were no ova or parasites in the stools A precipitin reaction for trichinosis (according to Roth) was negative The ESR was 129—142 mm/h Several blood cultures and examinations of the blood for L E cells were negative as was examination of blood and spinal fluid for viruses or complement binding factors Serum bilirubin level thymol turbidity serum alkaline phosphatase, prothrombin proconvertin time serum glutamic-oxaloacetic and serum glutamic pyruvic transaminases and serum electrolytes were within normal limits Serum electrophoresis showed

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369 sera from patients with chronic thyroiditis, adult myxoedema, thyrotoxicosis, atoxic, adenomatous goitres, L.E.D., Sjogren's syndrome and from blood donors were tested for cytotoxicity to thyroid cell cultures using the method of standardization described previously (6). The same sera were tested for complement-fixing, microsomal antibody, thyroglobulin antibody and CA-2 antibody. All sera containing the complement-fixing antibody were cytotoxic. Some sera were cytotoxic without containing the complement-fixing antibody, but no sera containing the complement-fixing antibody were non-toxic. The similarity in distribution between the complement-fixing antibody and the cytotoxic factor suggests identity. The distributions of thyroglobulin antibody and of CA-2 antibody were such as to suggest that no connection exists between these antibodies and the cytotoxic factor.

Thyroid cell cultures grown for more than 3 or 4 days after trypsinization develop resistance to the cytotoxic factor (1). No explanation of this phenomenon has been found. With an immune fluorescence technique applied to cell cultures and a Hashimoto serum containing the complement fixing antibody in a high titre, it could be shown that the cytoplasmatic fluorescence which was very bright in 16 hour old cultures became more and more faint in older cultures, and in 4 day old cultures no cytoplasmatic fluorescence could be demonstrated. This suggests that an antigenic deletion of the cultured cells takes place, and that the cells become insensitive to otherwise cytotoxic sera because the antigen corresponding to the cytotoxic factor disappears in 3—4 days of culture.

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aminations the physical examination had been normal but the white blood cell count had fluctuated between 7,000 and 42 000 and a differential count had shown 23 % eosinophils The hemoglobin value had decreased from 13 g % to 9 8 g % but had then spontaneously normalized There had been no occult blood in several stool specimens and the serum haptoglobin and serum iron values had been within normal limits The ESR had been slightly elevated There had at times been traces of albumin in the urine and sometimes the urinary sediment had contained 8—10 red or white blood corpuscles per visual field but the serum creatinine level had been normal Chest X ray and barium meal examination of the stomach had been normal

The patient had been treated with penicillin orally On the days just preceding admission the patient had difficulty in writing with his right hand and the left hand had appeared weak and fumbling He had also felt sore nodes on his neck and had noticed a red petechial rash on his body The patient had smoked at least 10 cigarettes per day for more than ten years

On admission physical examination revealed petechiae over all the body surfaces and also a vesicular exanthema which seemed to follow the vessels the lymph nodes were moderately enlarged the left hand was weak and fumbling in the ocular fundi there were inflammatory vascular changes surrounded by cotton wool exudates Examination of the heart the lungs and the abdomen was normal The blood pressure was normal

Hemoglobin concentration 12 g/100 ml Platelets 50 000/mm³ White blood cell count 42 000 with 8 % neutrophils 83 5 % mature eosinophils 0 5 % monocytes and 8 % lymphocytes Bone marrow aspiration showed 32 % eosinophils 25 % of which were metamyelocytes 25 % myelocytes and 50 % mature eosinophils there was no increase of immature neutrophils or blast cells but a moderate reticularcell hyperplasia the alkaline phosphatase activity of the leukocytes was rather high (60—115 scores) the eosinophilic cells were rather sparsely granulated and more or less pronounced plasma basophilia appeared also in the mature forms a few Charcot Leyden crystals were observed (A Hollender M D)

The serum vitamin B_{12} level was very high 2,900 pg/ml (normal range 175—960 pg/ml) Serum bilirubin level thymol turbidity, serum alkaline phosphatase prothrombin proconvertin time serum glutamic-oxaloacetic and serum glutamic pyruvic transaminases and serum electrophoresis were within normal limits (examined on the same day as the serum vitamin B_{12})

The ESR was 20 mm/h No L E cells could be found The serum creatinine value was elvated (3 1 mg/100 ml) and the urine contained albumin and an abnormal number of erythrocytes A plain film of the abdomen showed normal sized kidneys The precipitin reaction for trichinosis (according to Roth) was negative and repeated examinations of the stools failed to show ova or parasites

X ray of the chest was normal but the ECG showed marked abnormal changes of the S—T segment and the T waves Electroencephalogram gave a grossly abnormal EEG

Microscopic examination of skin lymph node and muscle biopsy revealed no pathological changes

The patient stayed in the hospital until he expired on May 8 1963 He was treated with prednisone 40—60 mg a day nandrolone phenpropionate and antacids Later digitalis and bendroflumethiazid were given

During the second week of hospitalization a pericardial friction rub was heard a few days later there was gallop rhythm and a systolic apical murmur which gradually increased in intensity The size of the heart increased considerably and later on a progressing cardiac decompensation with massive edema developed The liver increased gradually in size The lymphadenopathy disappeared

The serum creatinine level increased slowly up to 5—6 mg/100 ml the urine contained 5—8 % protein and many erythrocytes

The serum albumin level decreased to about 3 g/100 ml but otherwise the serum electrophoretic pattern remained normal

TABLE I Hematological changes (case 1)

Date	19/9	4/10	15/10	19/10
				†
WBC	14 000	7 000	28 000	39 000
Differential count				
Stem cells	—	6	20	—
Promyelocytes	—	6	3	—
Neut myelocytes	2	2	18	—
Neut metamyelocytes	3 5	—	5	—
Neut bands	8	7	2	—
Neut segmented	48 5	30	9	—
Eosinophils	19	22	16	—
Basophils	3 5	3	11	—
Hemoglobin (g %)	8 9	6 6	[1]7 1	5 2
RBC × 10[6]	3 9	2 5	[1]2 3	2 0
Platelets × 10[3]	—	44	40	7

[1] Two blood transfusions were administered during the period Oct 4 to Oct 15

moderate decrease of albumin level and slight increase of α_1- and α_2-globulins The ECG was normal as was the chest X ray (and the physical examination of the heart) X ray of the pelvis and extremities showed no skeletal abnormalities

The patient was treated with penicillin, streptomycin, prednisone 40—60 mg daily and analgesics During the second week of hospitalization he was given X-ray treatment of the right hip region (owing to severe pain), a total of 2 300 r The progress of the disease was characterized by severe, diffuse pain in the whole body, especially in the extremities sub febrile temperature sweats and general loss of health

Examination of the peripheral blood differential count included was made at least three times a week Representative values from the beginning, the middle and the end of the period of hospitalization have been compiled in table I As may be seen there was a constant mature eosinophilia blasts myelocytes and basophilic cells increased gradually in number and the anemia and thrombocytopenia progressed rapidly

The patient expired Oct 19 1963 32 days after admission that is 40 days after the first symptoms

Pathological findings (F Knutsson M D, Pathological Department I, Gothenburg) summarized

Petechiae over the whole body The heart was moderately enlarged with hypertrophy of the left ventricular wall and the myocardium was markedly pale The lungs were congested and infiltrated The liver and spleen were very large and weighed 3 050 and 1 350 g respectively The lymph nodes were of normal size with the exception of some slightly enlarged ones at the lung hilus Otherwise the macroscopic examination was normal

Microscopic examination of bone marrow, spleen liver lymph nodes kidneys and lungs revealed intense infiltration with mature eosinophils myelocytic eosinophils and blast cells with an overwhelming predominance of myelocytic eosinophils no vascular lesions or collagen tissue changes were seen The other organs were not examined microscopically

Case 2 A 29 year-old man was hospitalized on March 22 1963 The preceding four months he had during several periods of some weeks' duration experienced weakness muscular pains sweats fever abdominal distress and vomiting At ambulatory ex

(9) — is characterized by marked chronic or transient blood eosinophilia and involvement of many internal organs, especially heart lungs and nervous system, as well as of skeletal muscles, joints and skin The lesions in these organs consist predominantly of focal interstitial tissue degeneration and necrosis with mainly eosinophilic infiltration and of vascular lesions varying in severity from slight endarteritis to necrotizing arteritis During the acute phase, the macroscopic findings in the heart are minimal but microscopically there are cellular infiltrates, which are predominantly eosinophilic, in the endocardium and in the adjacent myocardium Small necrotic foci infiltrated by eosinophils are found in the myocardium At larger stages the heart is usually enlarged and displays thrombi and a greyish white thickening of the endocardium and extensive myocardial fibrosis Often the original endocardium cannot be recognized as such any more Underneath the thrombi, vascular granulation tissue may be found

The disease is thought to be of allergic or auto immune origin and related to Löffler s syndrome on one hand and to periarteritis nodosa on the other In adults the disease is usually fatal within months or a couple of years, often due to progressive cardiac insufficiency or irreversible vascular lesions (3 4 9 16)

Under the heading of disseminated eosinophilic collagen disease are included such probably related states as parietal fibroplastic endocarditis (Löffler s endocarditis) eosinophilic myocarditis allergic granulomatosis and angiitis (Churg and Strauss) and the syndrome of disseminated visceral lesions associated with extreme eosinophilia (Zuelzer and Apt) (3, 9, 16, 38)

Our second case is considered to be one of disseminated eosinophilic collagen disease It showed all the features characteristic of that disorder such as an intense eosinophilic reaction in the peripheral blood, a clinical course that was dominated by symptoms from the heart and — to a lesser degree — by symptoms from other visceral organs and from muscles and skin The pathological changes were of the type often seen in the later stages of the disease when the acute lesions have largely been replaced by fibrous tissue with few or no eosinophilic infiltrates (4)

Eosinophilic leukemia

During the course of the disease, our second case exhibited many features suggesting the presence of an eosinophilic leukemia the white blood count exceeded 80 000 and 85 % of the leukocytes were mature eosinophils while 2 % were eosinophilic myelocytes the serum vitamin B_{12} concentration was markedly elevated and the alkaline phosphatase activity of the leukocytes was within normal limits, there was a thrombocytopenic purpura the anemia progressed rapidly and the disease seemed to have a relentless self perpetuating course During the height of the eosinophilic leukemoid reaction the peripheral tissues were probably infiltrated with eosinophils and a necropsy examination at this time could easily have verified the diagnosis of eosinophilic leukemia Terminally the eosinophils disappeared and the blood picture resembled that of

The blood pressure increased considerably during the latter part of the illness

The neurological symptoms initially progressed but later regressed and a normal *EEG was obtained*

The retinal changes remained unaltered The petechiae disappeared The body temperature was normal except during the last week

Hematological changes

During the first ten days, the WBC increased to a maximum of 80,000 with 85 % mature eosinophils, 2 % myelocytic eosinophils, 10 % neutrophils and 3 % lymphocytes The *following* weeks, the number of white blood corpuscles decreased to a level of about 10—20,000 where it then remained for the rest of the illness period The percentage share of mature and immature eosinophilic cells decreased gradually at the same time as the number of mature neutrophils steadily increased The last day (May 8) the WBC was 14,000 and a differential count showed 0 5 % myeloblasts, *1 % myelocytes*, 97 % neutrophils, 0 5 % eosinophils, 0 5 % monocytes and 0 5 % lymphocytes

Repeated bone marrow examinations (A Hollender, M D) showed increasing eosinophilia with a maximum value of 69 % There was no shift to the left, on the contrary *the eosinophilic cells had hypersegmented* nuclei but, except for this, no abnormalities of the nucleus, the alkaline phosphatase ac*tivity remained normal*, when the last bone marrow examination was made on April 16 the number of eosinophilic cells had decreased to 29 % but except for this the picture was still essentially normal There was a moderate tendency to the development of a normocytic anemia, and the number of thrombocytes fluctuated between 24 000 and 160,000, the terminal values were Hb 7 4 g/100 ml and *thrombocytes 77 000 (May 8)*

The patient expired from severe heart failure and uremia

At *autopsy* (performed by L Schnurer M D , Pathological Department I Gothenburg) the heart was moderately enlarged with hypertrophy of the left ventricular wall Both atria were slightly dilated All the valves were normal The parietal endocardium of the apical region of the left ventricle and of the septal part of the right ventricle was dull, somewhat reddish and covered with fibrin Section of the myocardium showed *numerous greyish white, fibrous lesions* The coronary arteries appeared to be normal *Microscopically*, a 2 mm thick endocardial covering could be seen, near the lumen it consisted of fibrin, but closer to the myocardium it turned diffusely into a fibrous cell rich granulation tissue of an unspecified type, in several areas of the myocardium and often without endocardial involvement there were *irregular, sometimes* confluent fibrous lesions and in a couple of these areas there was fibrous obliteration and re canalization of vessels

In the kidneys the small arteries showed pronounced changes with severe thickening of the walls and concentric constriction of the *lumen mainly caused* by a *very pronounced* sub intimal fibrosis

The somewhat larger vessels in *the kidneys*, as was the case with the corresponding vessels in *the lungs, the spleen* and *the brain*, showed intimal fibrosis and fibrous obliteration with pictures of re canalization in some places *In the small lung* vessels, there were also some recent fibrin thrombi In the spleen and in the brain, the vascular changes were often combined with minor infarcts In the brain there were also multiple, partly confluent necrotic *lesions of different ages with out* provable injury to the vessel

Except for this the microscopic examination of the organs mentioned and of the liver and lymph nodes was normal The bone marrow was autolytically changed and therefore difficult to evaluate but it appeared *to* be normal *There were no* eosinophilic in filtrates There were no signs of polyarteritis nodosa

Discussion

Disseminated eosinophilic collagen disease — designated by Engfeldt and Zetterström

(9) — is characterized by marked chronic or transient blood eosinophilia and involvement of many internal organs, especially heart lungs, and nervous system as well as of skeletal muscles joints and skin The lesions in these organs consist predominantly of focal interstitial tissue degeneration and necrosis with mainly eosinophilic infiltration and of vascular lesions varying in severity from slight endarteritis to necrotizing arteritis During the acute phase, the macroscopic findings in the heart are minimal but microscopically there are cellular infiltrates, which are predominantly eosinophilic in the endocardium and in the adjacent myocardium Small necrotic foci infiltrated by eosinophils are found in the myocardium At larger stages the heart is usually enlarged and displays thrombi and a greyish white thickening of the endocardium and extensive myocardial fibrosis Often the original endocardium cannot be recognized as such any more Underneath the thrombi, vascular granulation tissue may be found

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Eosinophilic leukemia

During the course of the disease our second case exhibited many features suggesting the presence of an eosinophilic leukemia the white blood count exceeded 80 000 and 85 % of the leukocytes were mature eosinophils while 2 % were eosinophilic myelocytes the *serum* vitamin B_{12} concentration was markedly elevated and the alkaline *phosphatase* activity of the leukocytes was within normal limits, there was a *thrombocytopenic* purpura the anemia progressed rapidly and the disease seemed to have a relentless self perpetuating course During the height of the eosinophilic leukemoid reaction, the peripheral tissues were probably infiltrated with eosinophils and a necropsy examination at this time could easily have "verified the diagnosis of eosinophilic leukemia Terminally the eosinophils disappeared and the blood picture resembled that of

a mature neutrophil myelogenous leukemia

Actually, many cases reported as eosinophilic leukemia have shown clinical, hematological and pathological changes resembling those in our case 2 In nearly every instance, attention has been drawn to the maturity of the eosinophils, to the very high frequency of cardiac and other visceral symptoms and to pathological changes of a non-leukemic character (9) A minority of recent observes (6, 10, 33) have accepted these peculiarities as characteristic of eosinophilic leukemia but others have stressed the fact that the possibility of a leukemoid *reaction to a collagen or other disease* must be considered, even in cases with blast cells or terminal myeloblastic 'crisis''

Thus Engfeldt and Zetterström (9) considered it highly doubtful whether real eosinophilic leukemia had ever been *reported Bousser (3) in his careful* analysis of the 29 cases reported as eosinophilic leukemia in 1944—1956 found that 16 cases without terminal myeloblastic transformation and seven cases *with myeloblastic change had pro* minent clinical and/or anatomical features alien to leukemia but typical of disseminated eosinophilic collagen disease and he concluded that all these 23 cases were not leukemias but instances of dis seminated eosinophilic collagen disease the remaining six cases were *probably* true myelogenous leukemias but in two of these the eosinophilia was inconstant in two others mature and immature neutrophils predominated and in two cases essential data was lacking Thus Bousser was unable to find a single case of well-documented eosinophilic leu kemia in literature

Bousser (3) considered it impossible to decide whether a myeloblastic change *in cases of disseminated eosinophilic col* lagen disease should be regarded as a leukemoid reaction or as a leukemic process provoked by the collagen disease Hayhoe (16) interpreted the myeloblastosis as *illustrating* an extreme form of eosinophilic leukemoid reaction rather than a true leukemia Dameshek and Gunz (7) state that the difficulties of defining precisely what constitutes a leukemic process are particularly pronounced in the small group of possible *eosinophilic leukemias and that in many* of them the diagnosis must still rest on personal conviction rather than strict scientific criteria

Gross et al (14) and Winter (37) mention the possibility that an intense and constant eosinotactic stimulus may even *tually* cause a *neoplastic* leukemic trans formation Many other authors have stressed the fact that it is impossible to separate on morphological grounds a reversible (leukemoid) process from a *neoplastic (leukemic) process* (22, 26 29, 33, 37) Hematological changes such as sixty per cent eosinophilic myelocytes (37) or more than ninety per cent blasts in the peripheral blood (21) atypical cell forms (13, 31, 32 36, 37) and absence of alkaline phosphatase activity of the granulated leukocytes (18) have been reported in connection with leuke moid reactions

Gerhard (12) *maintains however*, that infiltration of immature leukocytes in tissues which do not have embryonal hematopoietic activity has not been

lescribed so far except in cases of leukemia Other authors, however, have pointed out that the cells of the reticulo endothelial system (which is to be found in practically all organs) have a latent and pluripotent possibility of developing into different blood cells, thus there is no essential difference between true leukemia and a leukemoid reaction pathogenetically or pathologically (22 37) Even a thorough post mortem examination may fail to differentiate between the two states (32, 33)

Thus the diagnosis of eosinophilic leukemia cannot be firmly established until all other causes of eosinophilia have been meticulously excluded (3, 7, 16)

In brief most authors — even if they are not quite convinced as to the existence of eosinophilic leukemia as a disease entity — consider it reasonable to give this title to a disorder having the general clinical hematological and pathological features of myelogenous leukemia but a *constant* and prominent increase of mature and immature eosinophils in the peripheral blood and bone marrow until death or terminal myeloblastic transformation *and a complete lack of any infectious parasitic neoplastic or collagen disease that might be responsible for the leukocytic proliferation* (3 7 14 16) Gross et al (14) also require proof of a neoplastic generalization of the eosinophilic cell infiltrates i e infiltrates also in tissues which do not have embryonal hematopoietic activity

Unequivocal examples of such a disorder are extremely rare if indeed they exist at all (3 16)

Since the work of Bousser in 1957 additional cases have been published under the heading eosinophilic leukemia Table II contains representative clinical hematological and pathological data of fifteen cases reported as eosinophilic leukemia 1956—1963 and includes our case No 1 The cases may be divided into the following four groups (listed in order, top to bottom in table II)

1 Five cases (Fadell Mallarme, Chen, Bentley, Thomas) with evident anatomical changes of the type characteristic of disseminated eosinophilic collagen disease and one case with suspected changes of this type (Boccato) Two of these (Boccato and Bentley) showed myeloblastic change, one case (Thomas) was associated with initial erythrocytosis (literature describes another case of that type (15))

2 One case with chloroma (Panoff), one case with lymphatic leukemia (Cei), one case with Stevens Johnson s syndrome (Ricci) and one case with bacterial endocarditis (Gerhard) Malignant states, dermatoses and severe bacterial infections belong to the diseases *which* may cause myelocytic or blastic leukemoid reactions

3 Two cases (Kienitz, Oehme) which cannot be evaluated because of the absence of necropsy examination

4 Four cases (Gross Sacréz, Papageogiou author s case No 1) with typical leukemic changes and no signs of an underlying disease capable of provoking a leukemoid reaction, the diagnosis of eosinophilic leukemia can not however be considered completely certain even in these cases because in Gross case the constantly mature cell picture in blood and bone marrow contrasted in a very remarkable manner

TABLE II Cases reported as eosinophilic leukemia 1956—1963 including the author's cas (three

Author Year	Age (yrs) Sex	Duration of symptoms¹	Clinical history and physical findings
Fadell 1957	27 ♂	— 6	A few short attacks of delusions, dizziness, vomiting and abdominal pain, low fever, pulmonary infiltrates, petechiae, splenomegaly, terminal tachycardia, gallop rhythm, and dyspnea
Mallarmé 1959	57 ♀	18 2 5	Several attacks of pneumonia, of bronchial asthma with urticaria and of fever, arthralgias, loss of weight, hepatosplenomegaly, lymphadenopathy, pulmonary infiltrations
Chen & Smith 1960	36 ♂	44 26	Paroxysmal cough, transient pleural effusions, congestive heart failure which gradually progressed in severity, cardiac catheterization data all in keeping with the diagnosis of endocardial fibroelastosis
Bentley et al 1961	5 ♂	7 7	Fever, petechial rash, coma and other neurological signs of short duration, nodules over both parietal bones, hepatosplenomegaly, lymphadenopathy, pulmonary infiltrations with short periods of laboured respirations
Thomas 1963	27 ♂	36 32	Paroxysmal cough, pericardial friction rub, epigastric and subxyphoid pain, progressive dyspnea, hemoptysis, cardiac murmurs, cardiomegaly, ECG changes, pulmonary infiltrations, hepatosplenomegaly, lymphadenopathy

cases ref 11 24 and 34 have been omitted)

Hematological findings[a]	Necropsy findings
Mature eosinophils in peripheral blood (86—55 %) marrow and other tissues No cellular atypies No blasts No anemia No thrombocytopenia Marrow 34—40 mature 8—3 % myelocytic and 12—7 % metamyelocytic eosinophils	*Heart* Large mural thrombus in left and right ventricle with destruction of endocardium and ingrowth of well vascularized immature fibrous tissue Within the myocardium were present large areas having loss of cellular detail surrounded by proliferating connective tissue densely infiltrated with mature eosinophils Many small vessel thrombi in the myocardium and spleen Moderate infiltration with mature eosinophils in most organs
Blood Eosinophilia (max 53 %) with up to 10 % myelocytes terminal values not stated Many atypical eosinophils No blasts Marrow 14 % mature (many atypical cells) 3 % metamyelocytic 10 % myelocytic and 9 promyelocytic eosinophils 16 myeloblasts	*Heart* Pericarditis In the myocardium numerous necrotic areas surrounded by mainly eosinophilic cellular infiltrates Vascular and perivascular infiltration with cells predominantly eosinophils Eosinophilic infiltrates into most organs with young eosinophils only in the bone marrow the spleen and perhaps the pancreas
Blood Mature eosinophils (80 50 %) Slight anemia No thrombocytopenia Marrow (figures in brackets refer to smears prepared from necropsy specimens) 18 % (15 %) mature 13 % (24) metamyelocytic and 16 (22 %) myelocytic eosinophils 6 % (6) myeloblasts	*Heart* Extensive myo-endocardial fibrosis and cardiac mural thrombosis Thickened intimal layers in many small arteries in the myocardium Many small vessels in the lungs appeared to have been occluded and recanalized Spleen lymph nodes and liver were infiltrated by numerous mature metamyelocytic and myelocytic eosinophils Similar eosinophilic infiltration in the pancreas and the kidneys
Blood Mature eosinophils 64 Myelocytic eosinophils 20 % No atypies Terminally change to principally a myeloblastic form" No anemia Thrombocytopenia Marrow 41—1 6 % mature 13 4— 3 % promyelocytic 13 0 % metamyelocytic and 17 0 % myelocytic eosinophils 0—8 7 % eosinophilic leucoblasts and 2 85 3 % *myeloblasts*	*Heart* Mural trombus of the left cardiac ventricle In the myocardium numerous petechiae several tiny areas of scarring numerous macrophages and active fibroplastic proliferation Varying degrees of blast cell infiltrate in lungs liver spleen kidneys urinary bladder and lymph nodes (Previous skin biopsy sheets of lymphoid cells and also many developing eosinophils which seemed to be coming from the reticulum)
Blood Initially erythrocytosis Mature eosinophils 82 24 %) Cellular atypies Anemia Thrombocytopenia Marrow The first two years only mature eosinophils and no blast cells *four months* before death hypercellularity manifested chiefly by intense proliferation of myeloid elements with an increase in eosinophils and eosinophilic precursors	*Heart* The entire apical portion and septal aspect of the left ventricle contained a *firmly adherent* well-organized mural thrombus blending into the scarred myocardium vessels interstitium and *epicardium* were infiltrated by eosinophils Nearly all organs *were infiltrated* by eosinophils but only the lymph nodes contained young forms

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Thomas 1963	27 ♀	36 32	Paroxysmal cough pericardial friction rub epigastric and subxyphoid pain progressive dyspnea hemoptysis cardiac murmurs cardiomegaly, ECG changes pulmonary infiltrations hepatosplenomegaly lymphadenopathy

Hematological findings[1]	Necropsy findings
Blood Mature eosinophils (3)—54 %) with cellular atypies terminally Eventually increasing number of blasts in the blood terminally 26% blasts Anemia. Marrow 5 % mature eosinophils 11% myelocytic eosinophils 14% pro-eosinophilic myeloblasts 4% hemocytoblasts and 10% erythroblasts	*Heart* Large diffuse adherent parietal thrombus in the left ventricle Focal myocardial lesions with massive infiltration of eosinophils or cells belonging to the eosinophilic myeloid series extending up to the affected endocardium and into the parietal thrombus Massive infiltrates of similar cells in practically all organs including the bone marrow No statement as to blasts in bone marrow or tissues No statement pertaining to the proportions between mature and immature eosinophils Cuffs of eosinophilic cells around the small vessels in many organs
Blood Mature eosinophils (31—2%) Constantly about 60% blast cells Anemia thrombocytopenia Marrow First 6 % mature eosinophils and 29 % eosinophilic paramyeloblasts (=myelocytes with pronounced degenerative features) and 61 % blasts later (one month prior to death) almost only blasts and only 10% eosinophilic myelocytes now without degenerative features	Subrural tumour chloroma according to macroscopic examination no microscopic examination of the tumour Leucosis acuta paramyeloblastica hepatis lienis et renum Anemia organorum omnium Dystrophia parenchymatosa myocardii hepatis et renum
Blood First 2% mature and 5 % myelocytic eosinophils later about 2% mature eosinophils and 90 lymphocytes Marrow Prominent increase of eosinophils and 7% lymphoblasts initially later not examined	*No autopsy* Lymph node biopsy showed diffuse infiltration by mature lymphocytes some lymphoblasts and a few eosinophils
Blood Mature eosinophils 50—26 and myelocytic eosinophils 21—6 % Marrow initially predominance of immature eosinophils but later (one year prior to death) 70% eosinophils, chiefly mature forms (with cellular atypies)	*No autopsy* Biopsies Liver normal Lymph gland the normal architecture destroyed by numerous myeloid cells many of which were eosinophils Skin Stevens-Johnson's syndrome
Blood Initially 53 eosinophils of which 9% were non-segmented young forms thereafter increasing number of myeloblasts and promyelocytes terminally 11% and 14% respectively No statement as to eosinophilic cells in later stages of the disease Marrow ninety per cent eosinophils twenty per cent of which were *myelocytes*	*Heart* Bacterial endocarditis (mitral valve) with purulent abscess of psoas muscle pericarditis (no parietal endocarditis) Liver spleen, lymph nodes skin and bone marrow infiltrated by immature myeloid cells most of the *granulated* cells being eosinophils Mature and immature eosinophils in the capillars of many organs
Blood Mature eosinophils (70—35%) high percentage of atypical cells. Moderate anemia No thrombocytopenia Terminally lymphocyte resembling cells with naked nuclei Marrow De	*No autopsy* Biopsy of lymph node inflammatory changes

Table II Cont

Author Year	Age (yrs) Sex	Duration of symptoms[1]	Clinical history and physical findings
Boccato 1960	82 ♀	— 2	Thrombophlebitis fever cough hepatomegaly pains in the lumbo sacral region skin eruptions increasing dyspnea (terminal) and edema
Panoff 1962	5 ♂	7 3	Weakness swelling and pains in the joints tumour of the left orbit, hepatosplenomegaly
Cei et al 1955	70 ♂	12 12	A few attacks of abdominal pain and diarrhea
Ricci Crassi 1959	28 ♂	36 36	Attacks of fever arthralgia abdominal pain and Stevens Johnson s syndrome cardiac murmurs hepatosplenomegaly lymphadenopathy
Gerhard et al 1958	73 o	4 $3^1/_2$	Dysphagia and skin eruptions (due to leukemic infiltrations) low fever
Kienitz et al 1956	13 o	4 $3^1/_2$	Weakness slight shortness of breath fever loose bloody stools pneumonia pains in the right arm (X ray showed circumscribed cortical bone cyst) lymphadenopathy splenomegaly

Hematological findings*	Necropsy findings
Blood Mature eosinophils (35—54%) with cellular atypies terminally Eventually increasing number of blasts in the blood terminally 26% blasts Anemia Marrow 5 mature eosinophils 11% myelocytic eosinophils 14% pro-eosinophilic myeloblasts 4% hemocytoblasts and 10% erythroblasts	*Heart* Large diffuse adherent parietal thrombus in the left ventricle Focal myocardial lesions with massive *infiltration of eosinophils or cells* belonging to the eosinophilic myeloid series extending up to the affected endocardium and into the parietal thrombus Massive infiltrates of similar cells in practically all organs including the bone marrow No statement as to blasts in bone marrow or tissues No statement pertaining to the proportions between *mature* and *immature* eosinophils Cuffs of eosinophilic cells around the small vessels in many organs
Blood Mature eosinophils (31—2%) Constantly about 60% blast cells Anemia thrombocytopenia Marrow First 6 % mature eosinophils and 29 eosinophilic paramyeloblasts (=myelocytes with pronounced degenerative features) and 61 blasts later (one month prior to death) almost only blasts and only 10 % eosinophilic myelocytes now without degenerative features	Subrural tumour chloroma according to macroscopic examination no microscopic examination of the tumour Leucosis acuta paramyeloblastica hepatis lienis et renum Anemia organorum omnium Dystrophia parenchymatosa myocardii hepatis et renum
Blood First 2 % mature and 5% myelocytic eosinophils later about 2% mature eosinophils and 90% lymphocytes Marrow Prominent increase of eosinophils and 7 % lymphoblasts initially later not examined	*No autopsy* Lymph node biopsy showed diffuse infiltration by mature lymphocytes some lymphoblasts and a few eosinophils
Blood Mature eosinophils 0—26% and myelocytic eosinophils 21—6% Marrow initially predominance of immature eosinophils but later (one year prior to death) 70 % eosinophils chiefly mature forms (with cellular atypies)	*No autopsy Biopsies Liver* normal Lymph gland the normal architecture destroyed by numerous myeloid cells many of which *were eosinophils* Skin Stevens-Johnson s syndrome
Blood Initially 53 eosinophils of which 9% were non-segmented young forms thereafter increasing number of myeloblasts and *promyelocytes* terminally 11% and 14 respectively No statement as to eosinophilic cells in later stages of the disease Marrow ninety per cent eosinophils twenty per cent of which were myelocytes	*Heart* Bacterial endocarditis (mitral valve) with purulent abscess of psoas muscle pericarditis (no *parietal endocarditis*) Liver spleen, lymph nodes skin and bone marrow infiltrated by immature *myeloid cells* most of the granulated *cells being* eosinophils Mature and immature eosinophils in the capillars of many organs
Blood Mature eosinophils (70 35 %) high percentage of atypical cells Moderate anemia No thrombocytopenia Terminally lymphocyte resembling cells with naked nuclei Marrow De	*No autopsy* Biopsy of lymph node inflammatory *changes*

Table II Cont

Author Year	Age (yrs) Sex	Duration of symptoms[1]	Clinical history and physical findings
Boccato 1960	82 ♀	— 2	Thrombophlebitis, fever, cough, hepatomegaly, pains in the lumbo-sacral region, skin eruptions, increasing dyspnea (terminal) and edema
Panoff 1962	5 ♂	7 3	Weakness, swelling and pains in the joints, tumour of the left orbit, hepatosplenomegaly
Cei et al 1955	70 o	12 12	A few attacks of abdominal pain and diarrhea
Ricci Grassi 1939	28 o	36 36	Attacks of fever, arthralgia, abdominal pain and Stevens-Johnson's syndrome, cardiac murmurs, hepatosplenomegaly, lymphadenopathy
Gerhard et al 1938	73 o	4 $3^{1}/_{2}$	Dysphagia and skin eruptions (due to leukemic infiltrations), low fever
Kienitz et al 1936	13 o	4 $3^{1}/_{2}$	Weakness, slight shortness of breath, fever, loose bloody stools, pneumonia, pains in the right arm (X-ray showed circumscribed cortical bone cyst), lymphadenopathy, splenomegaly

Hematological findings[a]	Necropsy findings
creasing number of mature (41—11%) and myelocytic (22—0%) eosinophils increasing number (48—83%) lymphocytes and atypical lymphoid elements impossible to tell apart exactly	
Blood Initially mature eosinophils 76%. The last six months no eosinophilia but 64% blasts Anemia Thrombocytopenia Marrow 60—27 mature and 5—21% young eosinophils the last six months almost only blasts and no increased number of eosinophils	*No autopsy*
Blood Excessive (95%) constant mature eosinophilia Atypical cells Anemia but no thrombocytopenia *Marrow* 84% *mature eosinophils* 11% myelocytic eosinophils and 0 5 % blasts	*Bone marrow* Loss of normal architecture 97% eosinophils (no statement as to the degree of maturity) Lymph nodes liver spleen lungs kidneys and the mucosa of the small bowel were infiltrated by mature and immature *eosinophilic* cells Charcot Leyden crystals in many organs Eosinophilic hyaline membranes in the lung alveoli Clots of partly deteriorating eosinophilic cells in the kidney capillaries
Blood Eosinophils *constantly more than* 50% atypical granular cells Eosinophilic myelocytes 8 Blasts 2% Anemia Thrombocytopenia Marrow 70 % blasts cells and 22% eosinophils chiefly mature forms	Spleen liver lymph nodes and bone marrow intensely infiltrated with myeloblasts many of which seemed to differentiate towards *the eosinophilic series* Here and there 40—50% *eosinophils* A few small vessels in the kidneys surrounded by hyaline cuffs
Blood Eosinophils maximum about 20 %. Atypical cells Maximum 12 *eosinophilic myelocytes* Terminally 100 % blasts Anemia No thrombocytopenia Marrow 3—10% mature eosinophils eosinophilic myelocytes 53—33% neutrophil myelocytes 17—35% blast cells 15% (Marrow biopsy *loss of normal architecture* most cells were composed of eosinophils with round nuclei)	Myeloid mataplasia with evident eosinophilia *in the liver* bone marrow lymph nodes tonsils and kidneys
Blood Constant eosinophilia maximum 32 only mature cells Successive increase of neutrophil *myelocytes* (maximum 26 %) and blast cells (maximum 20 %) Basophils maximum 11% Severe anemia and thrombocytopenia Marrow 47% eosinophilic cells of which more than ½ were myelocytes Atypical cells 30 blast cells 8% basophils There was alkaline phosphatase activity in the granulocytes Charcot Leyden crystals	Intense infiltration by myeloid elements with an *overwhelming* predominance of eosinophilic myelocytes in bone marrow spleen liver lymph *nodes* kidneys and lungs

which the patient has been observed When two values are given they are placed chronologically

Table II Cont

Author year	Age (yrs) Sex	Duration of symptoms[1]	Clinical history and physical findings
Oehme 1962	7 ♂	29 24	Fever, hepatosplenomegaly ECG changes suggesting myocardial lesion skin eruptions skeletal pains terminal orchitis cerebral shock and cardiac insufficiency
Gross et al 1955	7 ♂	1 ¹/₄	Short attack of abdominal pain fever, soft cardiac murmur, lymphadenopathy (slight) hepatomegaly terminally general pains and dyspnea
Sacrez et al 1960	13 ♀	4 4	Fever, thoracic and joint pain dyspnea pulmonary infiltrations minor hepatomegaly petechiae
Papageorgiou 1962	51 ♀	12 12	Weakness slight effort-dyspnea fever thoracic pain petechiae lymphadenopathy hepatosplenomegaly
Author s case I	41 ♂	1 1	Fever sweats thoracic pain later generalized pain

[1] The upper figure indicates the probable duration in months the lower figure shows the time during

ically and pathologically, there were no pre requisites for a non leukemic disease which could have provoked an eosino philic leukemoid reaction, but as a microscopic examination of the heart was not made the presence of a dis seminated eosinophilic collagen disease (parietal endocarditis) could not be ex cluded with certainty

The other case was considered a dis seminated eosinophilic collagen disease, the diagnosis was based on an intensive but transitional eosinophilic leukemoid reaction on multiple, focal visceral symptoms and on macroscopic and micro-scopic changes discovered at autopsy and characteristic of the disease in ques-tion In the final stage, the blood pic ture was dominated by an enormous relative *neutrophil* leukocytosis and a pronounced lymphopenia

A review of literature shows that a well documented case of uncomplicated eosinophilic leukemia has never been described In many cases however, hematological changes which have been impossible to differentiate from real' leukemia have been combined with clinical symptoms and pathological le sions characteristic of disseminated col lagen disease indicating that eosino philic leukemia and disseminated eosi nophilic collagen disease may be variants of the same (auto-immune?) syndrome

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with the clinically acute course of the "leukemia" and the possibility remains of an underlying disease killing the patient at the height of an eosinophilic leukemoid reaction, Sacréz' case showed a picture of an acute myeloblastic leukemia, but there was no information as to any considerable increase of immature eosinophilic cells in blood (maximum 4 % eosinophilic metamyelocytes and 4 % eosinophilic myelocytes) or bone marrow and in other tissues there were "young" eosinophils only in the liver, in Papageorgiou's case and in the author's case, no microscopical examination of the heart was made and it is therefore impossible to exclude a parietal fibroplastic endocarditis (that is a disseminated eosinophilic collagen disease) (4,20)

Thus in this review of literature, as has been the case in previous critical reviews, it has been impossible to find one single case of well documented, uncomplicated eosinophilic leukemia In a great number of cases features suggesting eosinophilic leukemia and so called collagen disease were simultaneously present, this fact arouses the question whether a common etiologic factor may cause both true leukemic proliferation and systemic collagen tissue disease The collagen diseases are considered to be of auto immune origin and according to Dameshek and Schwartz there may possibly be a relationship between leukemia and auto immunization and it remains to be proved whether abberrant immunological responses when continued or repeated on numerous occasions may eventually give rise to what are called neoplastic proliferations' (of the white cell forming tissues) (8)

Perhaps eosinophilic leukemia and disseminated eosinophilic collagen disease are *variants of the same autoimmune syndrome*, a collagen *and* hematopoietic-tissue disease presenting at times as a leukemia (with more or less discrete collagen changes) and at times as a collagen tissue disease (with more or less pronounced hematopoietic changes)

After completion of the editing of this work, a short statement of a case of eosinophilic leukemia with the following features has been published

A 5 year old boy, eight months duration hepatosplenomegaly, general lymphadenopathy, leukocytosis initially dominated by atypical lymphocytes but successive increase of eosinophils to a maximum of 40 %, complement fixation reaction against toxoplasmosis 1 30, 1 120 1 480, dye-test titer 1 250, 1 1,250, 1 6 250 anti pneumolysin titer 1 600 1 600 and 17 600 Autopsy revealed immature myeloid cells primarily eosinophilic leukocytes of varying degrees of maturity, infiltrating different organs such as bone marrow, thymus lymph nodes spleen, liver, lungs and myocardium (1)

The result of the serological examinations in this case indicate either an infectious disease (with eosinophilic leukemoid reaction?) or some disturbance of the immunological reactions

Summary

Two fatal cases of eosinophilia are described In one case, there was a *constant* eosinophilia and autopsy showed a massive infiltration of eosinophilic myelocytes in the bone marrow and the peripheral tissues including tissues which have no embryonic hematopoietic activity clin

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From the Medical Department (Head H Letman M D) Town and County Hospital Middelfart Denmark

Primary Endocardial Fibroelastosis in Three Siblings

By

J Stræde Nielsen

Endocardial thickening caused by increased collagen and especially elastic fibres has since Kreysig described the disease endocarditis foetalis (in 1826), been published under numerous names (38) The term now most commonly used is that suggested by Weinberg and Himmelfarb (37) endocardial fibroelastosis This histological picture can to a varying degree be found in systemic diseases such as glycogenosis (39) gargoylism (1 12) protein deficiency (18) tuberous sclerosis (9) and amyloidosis (38) also as the after effects of myocarditis (36) and myocardial infarction (31) Similar but however somewhat divergent histological pictures can be seen in fibroplastic parietal endocarditis with eosinophilia (7) and carcinoid (38) As demonstrated by Andersen and Kelly endocardial fibroelastosis is most often seen as a secondary phenomenon to other cardiac anomalies (1) or as an isolated pathological finding combined with considerable hypertrophy of the heart (19)

The last mentioned can most often be seen in newborn especially localized to the left chambers of the heart, but can also affect the right half of the heart and the valves Such cases have been reported in recent years with increasing frequency in both Scandinavian (4 5, 13 17 22 24) German and English literature (11, 12 26 30 38) and it has also become obvious that the disease occurs in siblings (tables I and II) The object of this paper is to publish an account of the appearance of primary endocardial fibroelastosis in 3 of 4 siblings

Case reports

Three siblings with primary endocardial fibroelastosis have been admitted to the medical department of the Town and County Hospital Middelfart during the last 7 years

Case 1 Pregnancy and birth normal birthweight 3 875 g The child was healthy until the 14th day when she was admitted owing to the sudden appearance of feeding problems vomiting diarrhoea pallor lassi

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TABLE I Histologically verified cases of primary endocardial fibroelastosis in siblings

Authors	No	Sex	Age at first symptoms	Age at death	Heart weight (g)	Comment	Siblings
Axtrup (2)	1	♂	1 year	$2\frac{1}{2}$ years	220	No 1 harelip	No 4 and No 5 healthy
	2	♂	1 year	2 years	250	No 2 atresia	
	3	♀	2 years	3 years	360	of the anus	
Dordick (10)	1	♀	4 months	4 months	70	—	—
	2	♂	Newborn	2 months	77		
Eyler et al (11)	1	♀	—	18 months	—	—	—
	2	♀	28 months	29 months	150		
Giertsen & Pande (14)	1	♂	Newborn	38 hours	23	Identical twins with aortic stenosis	—
	2	♂	3 days	26 days	65		
Greaves et al (15)	2	♀	4 months	6 months	52	Identical twins	No 1 healthy
	3	♀	$4\frac{1}{2}$ months	6 months	48		
Kelly & Andersen (19)	1	♂	Newborn	16 months	152	—	—
	2	♂	4 months	5 months	100		
	1	♀	4 months	5 months	—	Twins	—
	2	♀	4 months	4 months	—		
Pavlowich (25)	1	♀	22 months	22 months	150	Complete A—V block	—
	2	♀	5 months	5 months	64		
Rosahn (27)	1	♀	4 months	4 months	60	No 2 hypoplastic left kidney	—
	2	♂	4 years	$4\frac{1}{2}$ years			
Ullrich (34)	3	♂	7 months	8 months	115	Identical twins of triplets	No 1 healthy No 2 died 3 months old after short illness with cough and dyspnoea Third triplet healthy
	4	♂	9 months	9 months	120		
Vestermark (35)	1	♀	—	$3\frac{1}{2}$ months	—	Parents are first cousins	No 2 healthy
	3	♂	$1\frac{1}{2}$ months	2 months	—		
Weinberg & Himmelfarb (37)	2	♀	$3\frac{1}{2}$ months	$4\frac{1}{2}$ months	90	No 2 mild coarctation of the aorta	No 1 healthy
	3	♂	$2\frac{1}{2}$ months	$3\frac{1}{2}$ months	75		
Winter et al (40)	3	♀	5 months	5 months	—	—	No 1 and No 2 healthy
	4	♀	$1\frac{3}{4}$ months	2 months			
Zanker & Fisher (41)	1	♀	6 months	8 months	72	Myocardial scarring and luminal thrombosis	—
	2	♂	3 months	3 months			
Present cases	1	♀	$\frac{1}{2}$ month	1 month	70	—	No 2 cleft palate
	3	♀	$\frac{1}{2}$ month	$4\frac{1}{2}$ months	—		
	4	♀	1 month	$2\frac{1}{2}$ months	85		

tude, and dyspnoea Death occurred 19 hours after admission The autopsy demonstrated a *hypertrophic and dilated heart* with a weight of 70 g (normal weight 18 g) The ductus arteriosus was beginning to close and foramen ovale slightly open The endocardium was thickened and of a grey-white appearance, especially in the left ventricle Otherwise no macroscopical anomalies were found On microscopical examination *of the endocardium it was seen that* the thickening was caused mainly by an increase in the number of elastic fibres

Case 2 The pregnancy and birth ran a *normal course*, birthweight 4,000 g The child was healthy until the age of 14 days when she was admitted owing to the sudden appearance of pallor and dyspnoea with vomiting On admission a systolic murmur was found at the top of the epigastrium, the liver could be felt as far down as the umbilicus and the spleen was palpable X ray examination showed a considerable increase in size of the heart and ECG showed a right axis deviation, diphasic T_1 and T and high P_2 The child was transferred to the paediatric department of the Odense Town and County Hospital where she improved under treatment with digitalis and oxygen The systolic murmur in the epigastrium disappeared the liver diminished in size until it was 2 cm below the right curvature, the heart became considerably reduced in size, and the patient increased in weight She was admitted 4 1/2 months old to Dronning Louises Children s Hospital i Copenhagen, for further examination *Endocardial fibroelastosis was considered* as a possible diagnosis, but heart catheterization was unsuccessful and *the patient died 6 days after admission The autopsy* showed considerable hypertrophy of the heart both of the left and right ventricles and the endocardium was thickened and yellowy brown in colour, especially in the *left* ventricle otherwise no macroscopical abnormalities were found Microscopical examination of the endocardium showed well-defined fibrosis with large numbers of elastic fibres

Case 3 The pregnancy and birth were normal birthweight 4,300 g A few attacks of cyanosis occurred *shortly after birth 15 days old the* patient was increasingly cyanotic and later pale, with dyspnoea, and with sweating and in poor general condition An X ray examination showed considerable diffuse enlargement of the heart and the liver could be felt to be slightly increased in size The ECG showed right axis deviation, flat T_1 and T_2 *together with negative T in* V_1 *and* V_4 The child died just before the age of 2 1/2 months At autopsy the heart was found to be considerably dilated and hypertrophic with a weight of 85 g (normal weight 22 g), and the endocardium in both ventricles could be seen to be considerably thickened with a whitish colour *It was not possible to find other malformations* either in the heart or in the other organs Microscopical examination of the endocardium showed a considerable increase *in the number of elastic* fibres

Familial history

Both parents were healthy With the help of the Genetic Institute of the University of Copenhagen both parents' families have been studied without it being possible to find any relationship between the mother s and father's families (Mogens Hauge M D) The mother had not had any abortions or stillborn children *There are a total of 4* children, the *3* girls with endocardial fibroelastosis and a girl, no 2 in the row who is now 6 years *old She was at the age of two* operated upon *for palatoschisis with good* results and the child in April 1961 showed no signs of the disease ECG and X ray of the thorax showed normal conditions A male cousin of the mother died at the age of 8 months after dyspnoea of a duration of 2 days Autopsy was not carried out Apart from this there is no noteworthy disease in the family

Discussion

That the case reports referred to here and the cases shown *in tables I and II*

The fact that it is a systemic disease has been emphasized by some few authors, inasmuch as they have found adventitial perivascular hyperelastosis in the coronary vessels and other medium sized arteries (12, 27)

A total of 32 histologically verified cases of primary endocardial fibroelastosis in siblings are collected in table I and in table II an additional 16 presumed cases The hereditary nature of the disease can therefore hardly be doubted This presumption is also supported by the cases of twins and triplets summarized by Giertsen and Pande in which 1 of each pair was healthy and the others suffered from endocardial fibroelastosis (8 14 26, 34) There is found however, in literature publications of 2 cases of primary endocardial fibroelastosis in only 1 of two monozygote twins In one case the author (20 21) relied entirely upon clinical data In the other case (29) the other twin is still alive 7 years old and completely healthy It is therefore possible that the disease can be caused by exogenic factors or that the gene causing the disease allows itself to be modified or depressed by unknown non genetic factors The available facts with several cases in siblings no families with cases in two or several immediately following generations and comparatively similar sex distribution of the verified cases strongly suggest an autosomal gene There appears to be little information only on the lack of relationship between the parents (34 35) and only in one case has consanguinity been demonstrated (35) Considering the probable high frequency of the disease any greater frequency of consanguinity between the patients parents can hardly be expected Familial studies (table I) show in the majority of cases only slight variations within the various families with regard to the time of manifestation, but a difference of more than one year has been described in a few pairs of siblings As is known from other hereditary diseases, it appears that in patients with primary endocardial fibroelastosis other congenital anomalies appear with greater frequency than usual, both in patients themselves and amongst their siblings (tables I and II, 12)

The frequency of primary endocardial fibroelastosis is not known with any certainty Some consider that it is the most frequent (30) others the next most frequent congenital morbus cordis (16) In an autopsy material consisting of 10 000 adults and children 20 cases of endocardial fibroelastosis (32) were found and in an autopsy material consisting of children under 14 years 1 3 % were found with this disease (12) In an autopsy material over the appearance of congenital heart diseases it was found that endocardial fibroelastosis constituted from 2 6 % (6) to 7 % (19) of the cases There are found in literature single, often somewhat doubtful cases of primary endocardial fibroelastosis in adults (3 4 11) but normally the disease runs a fatal course early in childhood owing to cardiac failure About 80 % die in the first year of life, often after a short period of illness The importance of the disease with regard to the legal medical aspect has been repeatedly emphasized inasmuch as it is one of the causes of sudden death in children (12 16) Until recently the

TABLE II Presumptive and doubtful cases of primary endocardial fibroelastosis in siblings

Authors	No	Sex	Age at first symptoms	Age at death	Hearth weight (g)	Comment	Siblings
Fisher (12)	—	♀	Newborn	2½ months	67	—	Two siblings died previously in early infancy in a similar manner
	—	♀	2½ months	2½ months	93	—	Two siblings died suddenly in early infancy
Giertsen (13)	1	♂	—	18 hours	35	No 1 no microscopical examination of the endocardium	—
	2	♀	—	1 hours	35		
Linde & Adams (23)	—	♀	1 month	Alive 7 years old	—	Clinical diagnosis	One sibling died from endocardial fibroelastosis
Still (30)	—	♀	6¾ months	7 months	100	—	One sister with exactly similar history died 5 months old
Tuleczynska (33)	—	♂	—	17 months			
		♀		Alive one year old		Clinical diagnosis	—
Vestermark (35)	2	♂		16 months		No 4 clinical diagnosis	No 1 and No 3 healthy
	4	♂	-	Alive 2 years old			

comprise a nosologic unit can be accepted, possibly with the exclusion of the 2 cases with aortic stenosis (14). It was earlier considered that the cause of endocardial fibroelastosis was an infection in foetal life, a theory, which has now been superseded. The newer theories have been considered by many authors (1, 4, 26, 27, 31, 38) and are for cases secondary to other congenital heart anomalies considered mainly to be caused by anoxic and mechanical factors (1). Primary endocardial fibroelastosis must be considered to be caused by a developmental anomaly in the myocardium probably caused by a genetic factor (4, 31, 35). An increase in the endocardial thickening with age (19, 34) and electron microscopical studies, which show signs that the increasing endocardial thickening is caused by fibrin deposits, are considered by some as a sign that the primary seat of the disease is the myocardium (31).

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diagnosis of primary endocardial fibroelastosis has normally been made at autopsy, but with increasing frequency the diagnosis is being made in vivo (6, 23) by means of the characteristic findings of heart catheterization, angiocardiography, ECG, X ray of the thorax and general examination It appears in addition that the prognosis with intensive treatment with digitalis, diuretics, etc, together with possible talc installation in the pericardial sack is somewhat better than previously considered (23)

There is still a lack of definite knowledge of the aetiology of primary endocardial fibroelastosis One must therefore emphasize the importance of carrying out autopsies on children who die in infancy showing signs of congenital morbus cordis or without definite cause, and of familial studies in connection with the disease With this undoubtedly more cases will be discovered, and there will be a better basis with which to give advice to families with one or more cases of the disease (28)

One is thus on the way to defining a condition, probably caused by genetic factors, which can be separated from the heterogenic group termed congenital morbus cordis

Summary

Three cases of primary endocardial fibroelastosis are referred to amongst 4 siblings, and a summary of the familially occurring cases in literature is given The disease must be considered as being caused by an autosomal recessive genetically conditioned anomaly of the myocardium The pathological anatomical differential diagnosis and the aetiological theories are considered The disease appears to be more common and the prognosis somewhat better than earlier considered

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Survival in a Case of Acute Oral Chromic Acid Poisoning with Acute Renal Failure Treated by Haemodialysis

By

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This paper describes a case of acute oral chromic acid intoxication associated with gastroenteritis acute renal failure, and liver damage in which the patient survived after repeated treatments with the artificial kidney This seems to be the first case to be reported in which survival has occurred after dialysis The course was studied by estimation of the chromium concentration in the blood, urine and dialyzing fluid A short literary survey of the toxicology of chromium poisoning is also given in this communication

Survey of the toxicology of chromium poisoning

The ingestion of hexavalent chromium compounds usually leads within a few *minutes to* a couple of hours to abdominal pains vomiting of yellowish green gastric matter diarrhoea and intestinal bleeding (24) In many cases death occurs during the initial collapse (23) but even in patients who survive this stage lethal toxic damage may develop not only in the kidneys but in the liver as well 3 4 7 11 18 20 21 27 29 32) According to reports the primary damage in human kidneys arises in the tubules (4 11 20) although glomerular lesions have also been observed (7) In animals lesions may occur both in the glomeruli and in the tubules (12 14 15 19 25 26) Hepatomegaly without jaundice has been observed in a patient with acute poisoning (11) and fatty liver was found at autopsy in patients with both acute and chronic intoxication (4 17)

Fritz at al in 1960 described a case of acute chromium poisoning *in which the* patient was treated by dialysis but died (7) During dialysis the blood chromium level fell from 0 190 to 0 170 mg/100 g

Chromic acid and chromates may cause dermatitis in persons sensitive to the metal or salt and because of their oxidizing properties may also set up primary irritation (35) Inhalation of chromates leads to respiratory tract irritation as well *as to* characteristic ulceration and perforations of the nasal sep*tum (21)* Grave pulmonary damage results from the inhalation of massive amounts of chromic acid *steam* (22) and this has also been considered *to* be the underlying cause in certain cases of pneumoconiosis (17) allergic asthma (5 33) and bronchogenic cancer (2 6)

Chronic chromium poisoning may occasionally result *in a* systemic effect Lesions in

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drink was also vomited He had no burning sensation in mouth or throat and none of the fluid passed into his respiratory passages

At the hospital he was given sodium bicarbonate solution by the mouth He did not appear affected, but about an hour later a burning sensation arose in the epipharynx and behind the sternum He was transferred to the Dept of Otolaryngology, where slight redness and oedema in the anterior palatine arches was noted there were no corrosive lesions A radiograph of the oesophagus was normal

Vomiting attacks persisted for 24 hours and he had attacks of pain retrosternally and in the upper part of the abdomen for the first 2 days and frequent attacks of diarrhoea (about 3 l during the first few days) for 5 days Weber's test positive in the stool He was tired and uneasy, but had no attacks of cramp or signs of shock Temperature high during the first few days A lung radiograph and plain views of the abdomen were normal but radiographic examination of the stomach revealed thickening of the mucous membrane Large doses of antacids were given for the gastritis but food was withheld for 9 days because of the risk of oesophageal perforation On the day of admission the pH was 7 25 and the standard bicarbonate concentration 16 2 mEq/l in the capillary blood

The patient received over 2 days 1 800 mg of dimercaprol (BAL) intramuscularly divided into 6 doses he also received 1 g of calcium Sandoz in a 10 % solution intravenously 3 times a day during the first 48 hours at the clinic

During the first 24 hours 700 ml of urine containing protein and an abundancy of white but only a few red blood cells were passed Although 5 litres of fluid were given over 2 days the urinary output diminished rapidly to anuria which lasted for 16 days

The patient was transferred to the Medical Clinic B (Renal Clinic) and haemodialysis was undertaken on the 7th 10th and 12th days after the poisoning Urinary secretion gradually increased again and N P N had returned to normal 3 weeks later The kidneys were normal in size just over 2 months later Mild microscopical haematuria was still present after 3 months but no proteinuria or bacilluria were noted

Jaundice developed on the 2nd day after admission and was most pronounced on the 9th day, when a serum bilirubin value of 9 9 mg/100 ml was recorded The serum lactic dehydrogenase concentration (S LDH) was 6 600 units on the 3rd day and then began to fall Neomycin was administered in order to reduce the bacterial flora in the intestines and thus spare the liver The values returned to normal after 3—4 weeks Bromsulphalein loading showed a normal result after one month

A haemorrhagic diathesis was noted on the 8th—10th days with epistaxis haematemesis conjunctival bleeding and bleeding from operation wounds following cannulation The thrombocyte count was 66 000/mm^3 and the thrombotest value 34 % The bleeding tendency disappeared rapidly after the thrombocyte count and thrombotest had returned to normal

Anaemia developed and became increasingly severe The erythrocyte count fell from 5 1 mill to a minimum of 1 6 mill /mm^3 on the 33rd day after which the reticulocyte count increased again and at a control test after 3 months the erythrocytes numbered 4 7 million The sternal marrow was normal as were the leukocytes

ECG was consistently normal No mental confusion was apparent but at times the patient was apathetic Neurological *status* normal An EEC made about 1 month after admission showed a mild unspecific abnormality which later disappeared EMG consistently normal The patient was discharged after 6 1/2 weeks in hospital and resumed his work symptom free after a further 7 weeks Marked Beau's *lines* were present on the nails indicating that a serious condition had been present

Concentration of chromium in blood urine and dialyzing fluid

The samples were collected in vessels washed out with nitric acid and blood samples were withdrawn through a stainless cannula

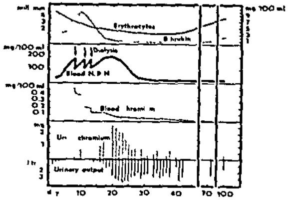


Fig 1

acid and 6—8 g for potassium bichromate (23) Symptoms of poisoning after oral administration of potassium bichromate in an amount as low as 0 5 g have been reported (27)

Chromium compounds can be absorbed through the respiratory tracts and alimentary tract and even seem capable of penetrating the skin (1, 8) Trivalent chromium is strongly bound to proteins while in the case of hexavalent chromium the bond is only loose and is unstable at a physiological pH Hexavalent chromium but not the trivalent compound, can penetrate the cell membranes of the erythrocytes (13) Trivalent chromium appears to constitute the main extracellular depôt from which soluble chromium in hexavalent form continuously diffuses through cell membranes by a metabolic process Inside the cell, it is reduced to the trivalent form, which becomes bound to the globulin fraction of the haemoglobin Chromium accumulates not only in the erythrocytes but also in the liver lungs, kidneys and spleen It is mainly excreted through the kidneys but may to a certain extent pass out with the faeces and through the skin

the gastrointestinal tract, the liver, the kidneys, the haematopoietic system, and the central nervous system have been described (3, 4, 7, 21, 28, 32)

Metallic chromium is biologically non-injurious In chemical compounds chromium has a valency of II, III, or VI The bivalent chromium salts are unstable and of little biological significance The trivalent chromium compounds do not dissolve readily in body fluids and do not as a rule produce serious damage in the body tissues In chromic acid ($H_2Cr_2O_7 = 2CrO_3 + H_2O$) and its salts (chromates and dichromates) the chromium has a valency of six These are the compounds which possess the greatest chemical activity, and which are most frequently used they are as a rule readily dissolved and because of their strong oxidizing properties they can cause severe irritation and may under certain circumstances have a toxic action on the body tissues They are considered to be 100 times more toxic than the trivalent chromium compounds and are those which give rise to industrial health problems The hexavalent chromium compounds are used among other things in the production of dyes and in plating The latter is an electrolytic process in which chromic acid occurs at a level of 20—40 %

The threshold limit value (MAC) for chromic acid and chromates in the air is 0 1 mg m³ A maximum allowable amount of 0 05 mg of chromic acid per litre is recommended for drinking water The lethal oral dose has been set as low as 1—2 g for chromic

Case report

Fig 1 gives the important data and the clinical course

A man of 30 with no previous history of illness had worked for 8 years — about 2 hours a day — in a wellventilated plating room without subjective or objective signs of chromium intoxication The plating fluid contained 300 g of chromic acid per litre and had a pH of 0 5 The patient was asked to empty the plating fluid from a bath on the first floor into a vat on the ground floor through a 5 meter long siphon having a diameter of 20 mm To start the flow he sucked the tube The fluid rushed suddenly through the tube and into his mouth and without thinking he swallowed it He rinsed his mounth with water immediately afterwards and about 5 minutes later he vomited yellow fluid Milk which he was given to

to chromium A mate working in the same environment was examined one month later and excreted 0 01 mg of chromium per litre of urine over 24 hours Earlier authors have as a rule found less than 0 1 mg of chromium per litre of urine in chromium workers (1)

It was not possible to ascertain the exact amount of chromic acid taken in by our patient on the occasion in question, but at an estimate he swallowed at least 50 ml of the solution (at least 15 g of chromic acid) Although some of this was lost by vomiting the dose undoubtedly exceeded the amount of 1—2 g mentioned in the literature as being fatal (23)

An important observation was the fact that absence of irritation of the mucous membrane or of corrosive lesions in throat and oesophagus does not preclude absorption and poisoning The same is the case in connection with *inhalation* of large quantities of chromic acid The deeper pulmonary structures may apparently be severely damaged while the nasal mucosa is only mildly hyperaemic (22)

The clinical course in our case with acute gastroenteritis toxic hepatitis and tubular nephritis somewhat resembles that of potassium chlorate and sublimate poisoning

The anaemia and short lived haemorrhagic tendency was of the type and severity usually seen in uraemic patients with acute renal insufficiency Whether the chromic acid had also affected the erythropoiesis or not is not clear

The following points of view on the treatment of severe chromium poisoning may be mentioned

Immediate administration of acid neutralizing substances followed by gastric lavage and administration of fluid to prevent primary shock have been recommended (23 24)

Prompt oral administration of ascorbic acid to poisoned rats has proved to be an effective antidote for chromate poisoning, similar treatment has therefore been suggested for human subjects (31)

Although our patient was treated at an early stage with dimercaprol (BAL) (12) this did not prevent the development of liver and kidney damage It is however, considered that BAL is not a sure remedy for counteracting chromium intoxication and nor is the treatment with sodium calciumedetate ($CaNa_2$ EDTA) of value because it gives no complexes with hexavalent chromium (7) Penicillamine has not been used as far as we know

The *administration* of large quantities of fluid and urea in order to produce polyuria and to accelerate renal excretion is, in our opinion an adequate *form* of therapy, provided the renal *function is* satisfactory In view of the risk of renal insufficiency — *which* occurred in our case — the urinary output and the body weight should be kept under observ*ation* for signs of fluid retention in connection with this treatment

Complicating renal insufficiency requires adequate therapy for this particular condition

Our results have confirmed previous reports to the effect that chromium is removed by haemodialysis (7) If forced polyuria cannot be achieved the latter treatment should be carried out as soon as possible after the poisoning has

The blood chromium level was determined by the method of Urone and Anders (34) except that the ashing was done in a sand-bath As the presence even of small amounts of iron disturbed the colour development the iron was removed as completely as possible The sensitivity of the method was estimated to be 0 5 μg per sample Amounts of 4—12 g of blood were used for analysis

The chromium level in the urine and dialyzing fluid was determined by Saltzman's method (30) The evaporation on a sand bath and oxidation were carried out in 100 ml Erlenmeyer flasks of pyrex glass with a broad neck As slight cloudiness was noted in nearly all the samples after colour development the solutions were centrifuged prior to the measurements in the spectrophotometer A Beckman Model DU with 1-cm absorption cells was used The sensitivity of the method was 0 5 μg per sample analyzed Amounts of 50 ml of urine and dialyzing fluid were used for analysis

The 24-hour output of chromium excreted with the urine was estimated on the basis of the concentrations in the urine, and the daily urinary output The patient was anuric *during the first 6 days From the 21st day* onwards only a small amount of the large daily outputs was analyzed, and consequently the estimated chromium excretion is not entirely accurate because of the variations in the chromium level at different times during the 24-hour periods

The chromium passed with the faeces was not measured

Fig 1 shows the results of the analysis The blood chromium level was measured *from the 7th day onwards* the blood samples being divided into serum and blood cells each of which were analyzed separately Calculated on the same quantity by weight the amount of chromium was 3—5 times greater in the blood corpuscles than in the serum The blood chromium level fell from 0 195 to 0 135 mg/100 g during the third dialysis treatment On the 100th day it was 0 005 mg/100 g blood

In the small amounts of urine obtained during the 7th to 9th days the chromium concentration was of an order as high as 7—9 mg/l It was around 2 mg/l during the early days of the polyuric stage and then showed a successive decrease A rough estimation of the total excretion of chromium in the urine between the 7th and 40th days gives a figure of 30—35 mg At a control test on the 100th day, the urine contained 0 13 mg of chromium per litre

In the dialyzing fluid, the chromium level was 0 08 mg/l during the first treatment with the artificial kidney, this means that a total of 24 mg of chromium was removed together with the dialysate

Control test Our dialyzing equipment is made of stainless steel and cellophane tubing is used as the dialyzing membrane No chromium was demonstrated in the dialysing fluid obtained at three treatments of uraemic patients without chromium poisoning In a patient with anuria, the concentration of chromium in the blood was roughly the same 0 005 to 0 006 mg/100 g — both before and after dialysis In a patient with acute renal failure who was dialyzed during the anuric stage less than 0 05 mg of chromium per day (with a urinary output of 1 200 ml) was passed during the polyuric stage

Comments

The patient had possibly been exposed to chromic acid mist for several years in connection with his work Prior to the present severe oral intoxication he had not undergone examination for exposure

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occurred, in order to reduce the risk of grave parenchymal damage resulting from the chromium intoxication

Summary

A case of survival after severe oral chromic acid poisoning is described The amount of chromic acid ingested was estimated to be 15 g or a dose greatly exceeding that mentioned in the literature as being fatal (1—2 g) The course was characterized by vomiting and diarrhoea, acute renal failure, liver damage, and haemorrhagic diathesis The concentrations of chromium in the blood, urine, and dialyzing fluid were studied

Treatment with BAL had no clinical effect An attempt to provoke polyuria with large amounts of fluid was unsuccessful as the patient became oliguric

Three dialysis treatments, which had been indicated because of uraemia, eliminated the chromium — the blood chromium level was reduced by 30 %, from 0 195 to 0 135 mg/100 g, during one dialysis and at another dialysis treatment 24 mg of chromium were removed in the dialysate To all appearances the patient made a complete recovery

Forced diuresis and/or dialysis treatment should be undertaken as soon as possible after the poisoning, in order to reduce the risk of serious parenchymal damage

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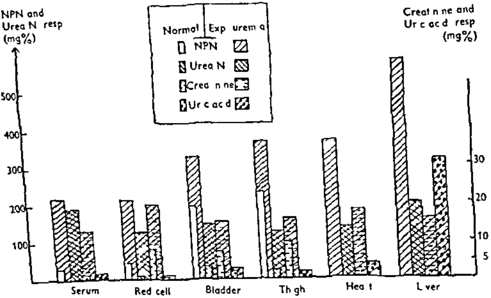
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F g 2

TABLE I Concentrat ons of N fract ons in the t ssues mg/100 g wet t ssue)

	Serum		Red cell		Bladder		Th gh		Heart	Li er
	Norm al	Uraem c	Normal	Uraem c	Normal	Uraem c	Nor al	Urae i c	Urae n c	Urae n c
NPN	31 30 7 1 7	(22 217 0 +11 (	(25) 45 9 +3 5	(15) 214 5 _37 4	(7) 198 2 ±12 0	(17) 329 7 ±14 6	(29) 235 6 ±7 (	(22) 366 8 ±13 7	(20) 367 5 ±9 5	(19) 376 1 +25 4
Ur a N	31 12 2 +1 1	20 190 2 +12 8	(23 11 8 +1 7	15) 125 7 ±10 6	5) 37 1 ±5 5	(17) 147 4 ±9 7	(21) 29 0 +3 7	(19) 129 6 ±5 7	(20) 136 9 ±8 8	(20) 200 7 ±12 5
Residual N	18	26 8	34 1	88 8	161 1	182 3	206 6	237 2	230 6	375 4

Results

The concentrat ons of NPN are shown in table 1 Bef re the intervention its mean level was 30 7 mg% in the serum and 45 9 mg% in the red cells The concentration was 235 6 mg% in the adductor and 198 2 mg% in the vesical muscle After a 3-day ligation of both ureters the concentration rose to about the same level in the serum and the red cells (217 0 and 214 5 mg% respec

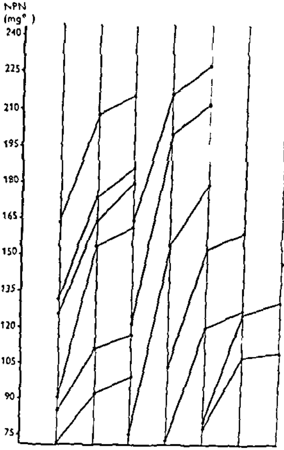


Fig 1 Each vertical line represents an interval of 24 hours

factor was believed to be the passage of a great amount of NPN and urea into the blood stream from the intra cellular space

In order to study the binding of *different NPN-containing substances in* the tissues and/or possible changes in their diffusibility, we induced acute uraemia in dogs by constricting both ureters for 3 days

Material and method

Forty dogs of both sexes with a body weight from 7 to 18 kg (average 10 5 kg) were used Blood was drawn from the femoral vein and a biopsy specimen taken from one of the adductor femoris muscles The fasting animals were narcotized with hexobarbitone sodium Median laparotomy was performed under sterile conditions, and both ureters were divided between two ligatures We tried to treat the bladder as carefully as possible (it was removed in some cases there and then) Most of the animals were weak retched and vomited when, 3 days after the operation they were *narcotized* once more We collected blood from the animals and took muscle biopsy specimens from the *intact adductor femoris, the rectus abdominis,* the bladder, intestine (from the middle and upper third of the ileum), heart and liver After washing the tissues with tap-water and then with doubly distilled water for a few seconds we dried them between sheets of filter paper for 30 to 40 seconds The samples were kept in Petri dishes at 2 to 4° C for not longer than 2 to 2 1/2 hours and then shredded with scissors triturated with liquid air and stored in doubly distilled water in the refrigerator After repeatedly agitating the suspensions, we centrifuged them 18 to 24 hours later, and analysed the supernatant fluid

We determined the NPN iodimetrically (9) after treatment with hypobromite, the *urea nitrogen was determined by means of* urease (1) with a suitable control The modified method of Popper et al (7) was *employed for the analysis of creatinine and a* modification of Brown s (5) procedure for that of uric acid Slight modifications of *these methods were made after comparison* with other techniques The composition of the sera and the red blood corpuscles was likewise analysed *The packed cell volume* was determined and the blood samples were haemolyzed with doubly distilled water for the purpose of erythrocyte analysis We measured the CO_2 combining power of the plasma with a volumetric apparatus of the Van Slyke type

Results obtained in respect of tissues are referred to 100 g wet weight or 100 ml intracellular water those in respect of sera to 100 ml serum or 100 ml serum water Mathematical mean values ($\bar{X}$) and standard deviations ($S_{\bar{x}}$) are given in the tables and diagrams

TABLE II Co cen ra ons of crea n ne and uric ac d n the t ssues (mg/100 g wet tissue)

	Serum		Red cell		Bladder		Th gh		Heart	Liver
	Normal	Ur e m c	Normal	Ur em c	No mal	Ur e n c	Norn al	Uraem c	Uraem c	Urae n c
Creatin ne	(20) 1 5 ±0 1	(14) 13 1 ±0 6	(19) 8 3 ±0 5	(13) 19 9 ±1 3	(5) 7 7 ±0 6	(10) 15 4 ±1 2	(16) 10 0 ±1 1	(15) 16 1 ±1 0	(15) 18 1 ±1 0	(14) 16 2 ±1 0
Ur acid	(19) 0 97 ±0 2	(14) 1 62 ±0 2	(9) 0 93 ±0 3	(9) 1 26 ±0 2	(6) 2 53 ±0 4	(9) 3 20 ±0 6	(15) 1 29 ±0 1	(14) 2 10 ±0 4	(15) 4 11 ±0 5	(14) 31 7 ±4 0

TABLE III Con entra ons of n acellular urea N

	B adder	Th gh	Heart	L ver	Red cell	Serum
Normal	62 1	40 7			16 6	13 1
U aemic	183 0	146 6	163 6	279 0	177 0	204 6

No e The mean values of table I are here referred to in ra ellular wa er (mg/100 ml) The total wa er con en of he issues was taken to be 78 that of the ery h ocytes 71° he extrac llular compa men n he ves cal muscles of heal hy dogs was supposed to amount to 25° n the h gh o 10 and ha of u aem c issues to 35 . The serum val e compu ed for a wa er con en of 93 is g en for he sake of comparison

rate of f rtl er rise is much slower once eq l l rium l as l een reached

W th a v ew to present ng a clearer p ct re of intracell lar quant tat e con l t ons we l ave referred tl e mean values of urea N n table I to intra cell lar water and the result ng data are shown n tal le III and f g 3 As has already been noted t is s ggested by certa n a tl ors 4) tl at tl e cells and tl e pla ma of pat ents with renal d seases conta n non-d ff is l le urea — wh ch wo ld expla n why the eff c ency of d alys s s often less than expected Aga n otl er workers (12) ma nta n tl at a relat e ncrea e in tl e amount of non d ffus ble urea accounts for the post-d alyt c level of urea being h gher in the muscles than in the serum

Data such as are sho vn in table III make it clear that no such theories are necessary in cases of acute uraem a The observed phenomena are suff c ently ex pla ned by the above ment oned grad ent l ver → serum → tissues This not w thstand ng the ex stence of a bound form of u rea is st ll poss ble It was found in prel m nary experiments that uraem c muscles conta ned a few mg% of nond ffu l le rea N

An analys s of the ava lable values determ ned before the construct on of

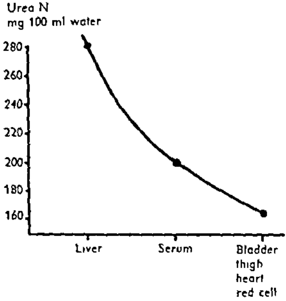


Fig. 3

tively), while the NPN level of the tissues varied between 330 and 370 mg%. The liver was an exception inasmuch as its NPN content rose to 576.1 mg%.

The concentrations of urea-N are likewise listed in table I. Before the intervention, the mean level of urea-N in the serum and the red cells was about 12.0 mg%, that in the vesical muscle and the adductor femoris 37.1 and 29.0 mg% respectively. In a state of uraemia, the urea N level was lowest in the erythrocytes (125.7 mg%). It was 190.2 mg% in the sera, and between 130.0 and 160.0 mg% in the tissues. The liver was an exception also in this case: its content of urea N amounted to 200.7 mg%.

Also the values for *residual N* (i.e. the difference between NPN and urea-N) are shown in the table. Its mean level in intact dogs is 18.5 mg% in the serum, 34.1 mg% in the red cells, 161.1 mg% in the vesical muscle and 206.6 mg% in the adductor femoris. It rose after ureteral ligation to 26.8 in the serum, 88.8 in the red cells, 182.3 in the vesical muscle, 237.2 in the adductor, 230.6 in the myocardium and 375.4 mg% in the liver.

The concentrations of *creatinine* and *uric acid* are listed in table II. The mean level of creatinine was considerably higher in the tissues than in the serum both in intact and in uraemic animals. The normal level of uric acid is about 1.0 mg% in both serum and red corpuscles, and higher in the other tissues examined. As is evident from the table, the uraemic animals showed about ten times as much in the liver as in the other organs.

Discussion

We examined in the present experiments the NPN containing substances found in the various tissues of uraemic animals. Tissues — owing to their bulk — proved to be important depots of such substances.

1. *Urea nitrogen.* Its concentration in the serum was lower than in the liver but higher than in the other tissues examined. It would seem that urea synthesized by and reaching its highest concentration in the liver, diffuses into the plasma and passes therefrom into the tissues. This process is modified by means of dialysis in human subjects since it is the serum level which diminishes most promptly and most markedly in the course of dialysis. The concentration being high in the intracellular compartment and low in the serum after the termination of dialysis, the serum level rises rapidly during the next few hours if anuria and oliguria persist the

itatively but a negligible portion of the NPN content The level of creatinine seemed to rise in uraemia more than that of uric acid

According to certain authors (6 11), creatinine diffuses freely in the organism This may hold for creatinine infusion, but our experiments showed that, under normal conditions the concentrations in the tissues and the red corpuscles were 4 to 5 times higher than those in the serum It is noteworthy from the viewpoint of the pathomechanism that 3 days after ureteral ligation, i e after a considerable rise of values the level of creatinine was but 50 per cent higher in the tissues than in the serum (table II) This phenomenon is presumably connected with the known fact that permeability increases in uraemia

diffusing from the tissues in healthy animals, and that the diffusibility of creatinine is undoubtedly and that of urea presumably increased in experimental uraemia

The results of the experiments justify the conclusion that the cause of the rapid rise in the blood level of urea during a few hours following haemodialysis in human patients is that the urea passes into the serum not merely from the liver, but from the intracellular space of other tissues as well The concentration of urea becomes lower during haemodialysis, and it is only after its termination that equilibration takes place between the urea level *in the serum* and that in the intracellular compartment

Summary

Uraemia has been induced in dogs and the concentrations of NPN, *urea N* creatinine and uric acid in certain tissues in the red blood cells and in the serum have been examined It was found that under physiological conditions the concentration of all substances examined was everywhere higher than *in the serum* the actual level depending on the test substance and the tissue examined The situation remained unchanged under uraemic conditions for all substances but urea N in respect of which the concentration showed the following gradient liver > serum > tissues

The diffusibility of urea and creatinine is discussed It was found that only a part of these substances is capable of

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the ureters shows that the normal amount of urea N was essentially the same in the red corpuscles and the serum If however, the values are referred to water content the concentration is 16 6 mg% in the erythrocytes against 13 1 mg% in the serum This is in accordance with the data of Ralls (8) who found the mean ratio between red cell water urea and plasma water urea to be 1 14 It is worthy of note that the normal concentrations of urea N in smooth and striated muscles are 37 0 and 29 0 mg%, respectively which if referred to cell water gives 62 0 and 41 0 mg% against about 13 0 mg% in the serum

The computation of this serum value is based on the assumption that the inhibition of urine discharge for an hour results in an equilibrium between tissues and serum If the maximum quantity of urea N produced per minute by a healthy dog of 10 kg is one mg and if the water space amounts to 78 % the serum level would rise in an hour by 0 8 mg% only i e to 13 mg% Since with a similar calculation 13 mg of creatinine is produced per hour in a dog of 10 kg and it is distributed over 78 % of the body weight the initial level of 1 5 mg% would rise to not more than 1 7 mg% after an hour

The values found in the red corpuscles do not in themselves explain these figures and the problem requires further investigation

It follows from the foregoing that the erythrocytes of healthy animals contain less their examined muscles more non diffusible urea This would explain the excess urea found by Schackman et al (12) in the muscles The high serum values made it impossible to estimate the urea binding capacity of the tissues under the present experimental conditions It is nevertheless safe to assume that it does not remain unchanged since a relatively high urea concentration in the tissues cannot be explained by diffusion from the serum unless the animal is suffering from acute uraemia In uraemia i e at a time of increased protein catabolism the amount of bound urea may — though only in a relative sense — become less in the tissues on account of the high rate of production in the liver

2 *Non protein nitrogen* The concentration of NPN is known (2 3) to be considerably higher in the tissues than in the serum under both normal and uraemic conditions Red cells were exceptional also in this respect Although their NPN level in healthy dogs was higher than that of the serum (but still considerably lower than that of other tissues) the concentration became equal in the serum and the erythrocytes after a constriction of the ureters

3 *Residual nitrogen* This is another instance of the special behaviour of the red blood cells Experimental uraemia raised the level of residual N in all organs examined While the rise was slight in the muscles it was more than twice as much in the erythrocytes

4 We determined the amounts of *creatinine* and *uric acid* in order to be able to study the level of residual N in the tissues more thoroughly It was found that while the concentration of both substances rose in the serum and the tissues examined they constituted quan

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From the Medical Service I Sahlgren's Hospital University of Goteborg Sweden

The Effect of Poldine, Glycopyrrolate and 1-Hyoscyamine on Gastric Secretion of Acid in Man

By

G Dotevall, G Schroder and A Walan

Recent years have brought a change in views on anticholinergic treatment of peptic ulcer disease The aim nowadays is to adapt the treatment individually to the patient The guiding principle here is to begin with an average re commended oral dose 3—4 times a day before meals and at night This dose is then progressively increased until in the course of a few days side effects are observed The most common of these is dryness of the mouth but blurring of vision palpitations constipation and difficulty of micturition may also occur When such symptoms appear the dose is decreased and the highest dose tolerated without side effects is used In most patients a slight feeling of dryness in the mouth is allowed to remain The dose arrived at in this way is called the optimal effective dose (OED) (17) The OED thus varies from one patient to another and ac cording to the preparation employed With optimal doses of anticholinergic drugs there is an inhibition of basal secretion, as well as of secretion stimu lated after food histamine and insulin hypoglycaemia (1, 2, 5, 6 10 11, 12, 14)

The purpose of the present investiga tion was to study the effect on gastric secretion of two recent synthetic drugs poldine and glycopyrrolate, and also to examine the effect of 1 hyoscyamine given in tablets with sustained release, Duretter® tablets (1 hyoscyamine Du retter tablets were supplied by AB Hassle, poldine by Beecham Research Labora tories Ltd and glycopyrrolate by AB H Lundbeck) In some of the patients investigated the effect on the circulation of poldine and 1 hyoscyamine was stud ied The results of these investigations have been published (13)

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Studies of the gastric secretion in patients with peptic ulcer selected at random were made while they were receiving no anti

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TABLE I Basal secretion of total acid in mEq/hr before and during treatment with optimal effective doses of poldine, glycopyrrolate and l hyoscyamine in tablets with sustained release The drug has been given 2 hours before beginning of the investigation

Type	No of pat	Dose (mg) (mean & range)	Total acid output in mEq/hr (mean ± SE) Before treatment	During treatment	% inhibition	Degree of significance
Poldine	14	7 9 (4–10)	7 8±1 9	3 8±0 7	51	P < 0 05
Glycopyrrolate	9	2 5 (1–3)	7 7±1 9	3 5±0 9	67	P < 0 05
l hyoscyamine tablets with sustained release	14	0 8 (0 6–1 0)	4 3±1 1	1 3±0 3	72	P 0 05

cholinergic drug and also while receiving poldine, glycopyrrolate or l hyoscyamine tablets with sustained release, Duretter" tablets

The patients were examined at 8 00 a m after being without food for at least 12 hours

A Levin tube of 3 mm internal diameter was passed into the stomach through the nose The patient was sitting in a semi upright position He was instructed not to swallow the saliva Continuous draining of the stomach was carried out by suction at a sub atmospheric pressure of 50 mm Hg Extra suction was applied by means of a syringe in order to prevent stagnation of gastric juice in the tube After removing the residual secretion from the stomach and determining that the tube was passing gastric juice satisfactorily two 30 minute collections of basal secretion were made after which histamine was given Thirty minutes before the commencement of histamine stimulation 100 mg of the antihistamine (Antazoline) was given by intramuscular injection

Histamine was administered subcutaneously in a dose of 0 024 mg of histamine hydrochloride per kg of body weight According to Kay (9) this dose produces maximal stimulation of gastric secretion of acid The gastric secretion was collected in 15 minute samples over a period of one hour The pH was determined potentiometrically in a Beckman pH meter, and the gastric juice was titrated with n/10 NaOH until pH values of 3 0 (free HCl) and 8 0 (total HCl') were reached The pH of each sample was measured, as well as the volume and concentration of total hydrochloric acid The results are given as mEq/hour total HCl and in pH taken to the nearest 0 05 unit

Two of the drugs to be tested poldine and glycopyrrolate, were given 4 times daily usually starting with a dose of one tablet containing 2 mg poldine or 1 mg glycopyrrolate The l hyoscyamine Duretter tablets were given 3 times daily on account of their long duration (4) One Duretter tablet contained 0 2 mg l hyoscyamine sulphate The dosage was increased every second or third day by one tablet per dose until side effects appeared When side effects appeared the dose was reduced to the next smaller dose at which most of the side effects disappeared The only side effect allowed to remain was a slight dryness of the mouth The dose was then maintained at that level for the test The last dose was given two hours before the beginning of the basal collection The order in which the gastric secretion tests with and without anticholinergic drugs were made was varied at random

The significance of differences between groups was tested with Student's t test and differences below the 5% level were regarded as significant

TABLE II Gastric secretion of total acid in mEq/hr after maximal histamine stimulation before and during treatment with optimal effective doses of poldine glycopyrrolate and l hyoscyamine in tablets with sustained release The drug has been given 3 hours before beginning of the investigation

Type	No of pat	Dose (mg) (mean & range)	Total acid output in mEq/hr (mean ± SE) Before treatment	During treatment	% inhibition	Degree of significance
Poldine	14	7 9 (4–10)	28 6±3 0	17 8±2 0	38	P < 0 05
Glycopyrrolate	9	2 5 (1–3)	28 4±2 2	15 7±2 3	45	P < 0 05
l hyoscyamine tablets with sustained release	14	0 8 (0 6–1 0)	24 1±3 1	15 5±2 3	40	P < 0 05

Results

1 *Basal secretion*

The basal secretion was studied in 14 patients before and during treatment with poldine In all patients the acid secretion decreased during poldine treatment (table I) (P < 0 05) The average decrease was 51 %

Ten patients were treated with glycopyrrolate Nine of these showed a marked reduction of HCl secretion, while the remaining patient showed an unchanged acid secretion during treatment The average decrease of basal secretion in these patients treated with glycopyrrolate was 67 % (P < 0 05)

Fourteen patients were treated with *l hyoscyamine in* Duretter tablets The HCl secretion decreased during treatment in all patients The average decrease was 72 % (P < 0 05)

2 *Secretion after maximal histamine stimulation*

All the patients except one studied in respect of basal secretion were also examined after maximal stimulation with histamine The optimal effective dose of the anticholinergic preparation was administered 3 hours before the histamine stimulation

Fourteen patients were examined before and during treatment with poldine In 2 of the patients the secretion was unchanged during anticholinergic treatment while in 1 patient the secretion increased slightly With the other 11 patients, an obvious reduction in HCl secretion was noted after maximal histamine stimulation The average reduction in secretion in all the patients examined was 38 % (p < 0 05)

Fourteen patients were treated with l hyoscyamine Duretter tablets, and 9 patients with glycopyrrolate Secretion after maximal histamine stimulation was reduced in all these patients The average reductions were 40 % and 45 % respectively (P < 0 05 and P < 0 05)

The side effects were all of parasympaticolytic nature and were similar for all drugs used The most common were dryness of the mouth and blurring of

vision One patient had difficulty in passing urine, which symptom disappeared when the drug was withheld

Discussion

There are various methods of evaluating the effect of anticholinergic drugs on basal secretion According to Sun (15), an anticholinergic preparation is effective as long as the pH value of the gastric juice is $\geqslant$ 4 5, since the pepsin is essentially inactive at higher pH values The secretion in mEq per unit of time is more informative as it furnishes data depending on volume as well as acidity With all three anticholinergic preparations tested a significant reduction in basal secretion was obtained Previous investigators have also shown a significant reduction with poldine (5, 6, 10, 11) and glycopyrrolate (1,1 6) Comparative studies of the effect on gastric secretion of the synthetic anticholinergics, poldine and glycopyrrolate, and of l-hyoscyamine are of both theoretical and practical interest The former consist of quaternary ammonium compounds incapable, in therapeutic doses, of penetrating the blood-brain barrier, while the latter represents the most active part of atropine, with the property of penetrating this barrier As shown in tables I and II the inhibition of gastric secretion obtained by the administration of the new anticholinergic drugs 2—3 hours before the investigation is of the same magnitude as with l-hyoscyamine Nor were any important changes found on circulation and respiration of prolonged treatment with poldine and l hyoscyamine respectively (13)

According to Sun (15), the synthetic anticholinergic drugs are to be preferred, particularly on account of their longer duration as compared with atropine The same view is held by Mitchell et al (11), who state that the effect of poldine has a duration of at least 8 hours, whereas that of atropine is considerably less When l-hyoscyamine is administered in tablets with sustained release, Duretter tablets, the effect is prolonged Investigations have shown a reduction of basal secretion during a period of at least 8—9 hours with tablets of this type (4) On account of the delayed absorption, the dose administered becomes approximately twice as large as with administration of the usual type of tablets (4)

Perorally administered anticholinergics have proved to have an effect even after maximal stimulation of the gastric secretion (1, 14) All the preparations tested here gave a reduction of acid secretion of about 40 % after maximal histamine stimulation Other investigators report similar results in respect of poldine (14) and glycopyrrolate (1) Treatment with anticholinergics can reduce to normal the increased secretion that is very often found in peptic ulcer disease In 21 patients with duodenal ulcer in the material reported on here, the secretion diminished from 33 2 mEq/hour after maximal histamine stimulation without treatment to 21 0 mEq/hour during anticholinergic treatment The secretion in a control material investigated at the same laboratory was 22 mEq/hour (3) The cause of the reduction in acid secretion during anticholinergic treat

ment is probably a diminished sensitivity to stimulation in the parietal cells As a rule, the change is quickly reversible in short term treatment, and as early as 12 hours after administration of the dose the secretion is back at the same level as before treatment (14) On the other hand prolonged treatment with optimal do es of anticholinergics may result in lower secretion even several days after the last dose (6) It may be possible that this depression is a result of a decrease in the parietal cell population consequent on diminished nervous stimulation Earlier investigations have shown that just as with basal secretion increasing doses of atropine give an increasing inhibition of histamine stimulated secretion (12)

In animal experiments (8) it has been shown that maximal secretory response after histamine was unchanged after vagotomy The amount of histamine required to produce the maximal response however was much greater after than before vagotomy, indicating that the parietal cells were less sensitive

In man Gillespie and Kay (7) have shown that after surgical vagotomy maximal histamine stimulation brings a decrease in acid secretion of 40—50 % If this is due to reduced sensibility in parietal cells and/or a reduction in the parietal cell mass is still an open question

Summary

1 The effect of three different anticholinergic preparations was studied in patients with peptic ulcer Apart from slight dryness of the mouth no side effects were accepted For each patient the basal secretion and the secretion after maximal histamine stimulation were investigated before treatment and 2 and 3 hours respectively after administration of the optimal dose

2 The synthetic poldine and glycopyrrolate, as well as 1 hyoscyamine in tablets with sustained release inhibited basal secretion of HCl The reduction was 51 % 67 % and 72 % for poldine, glycopyrrolate and 1 hyoscyamine respectively

3 Secretion after maximal histamine stimulation was also inhibited by the three preparations tested The reduction was 38 %, 45 % and 40 % for poldine, glycopyrrolate and 1 hyoscyamine respectively

4 The two synthetic anticholinergics tested showed no advantages over 1 hyoscyamine administered in tablets with sustained release

5 Various reasons for the reduced secretion of HCl during administration of anticholinergic drugs are discussed

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Effect of Short-term Hypoxaemia on the Potassium and Sodium Metabolism and on Cardiac Contraction

By

Vlastimil Jezek Alois Olrednik Severin Daum and Ludmila Kroužkova

The effect of respiratory insufficiency and its two components — reduced oxygen saturation of arterial blood and hypercapnic acidosis — on the development of heart failure in patients with cor pulmonale has repeatedly been discussed

Signs of heart failure in these patients are accompanied by changes in electrolyte metabolism particularly in potassium exchange Changes in potassium metabolism caused by acidosis are sufficiently well known Less well delineated are metabolic changes due to reduced oxygen saturation of arterial blood

Our aim was to find out whether even short term and only moderately reduced saturation of arterial blood (without a shift of the pH of blood towards acidosis) causes changes in the sodium and potassium levels and whether patients with cor pulmonale in chronic bronchitis respond in the same way as healthy subjects Next we investigated whether a short term hypoxaemia changes the course of cardiac contraction

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Material and methods

A total of 11 patients with chronic cor pulmonale in chronic bronchitis and emphysema were examined all showing in the blood gas analyses a decreased oxygen saturation None of these patients suffered from hypercapnia (pCO_2 was lower than 42 mm Hg) or acidosis (pH of arterial blood was above 7 35) and none had signs of cardiac failure Twelve healthy subjects were examined in the same way In the course of the investigations the patients were not subjected to any treatment

Examination procedure The subject collected his urine on the eve of the examination from 7 p m till 7 a m and on the day of the examination from 7 a m till noon and from noon till 7 p m and then again during the following night from 7 p m till 7 a m In the early morning an arterial puncture using Cournand s needle was carried out, subsequently the patient was connected to a Metabograph (Fleisch) After 15 min at rest blood was withdrawn from the artery for the estimation of electrolytes and blood gases and the patient inhaled (while his ventilation was recorded graphically) for an average of 45 min a gas mixture with normal CO_2 content a reduced oxygen content (14—18 %) and an increased nitrogen con

4 DOTEVALL G & WALAN A The effect of a new l hyoscyamine tablet with sustained release on gastric secretion of acid in man In preparation
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TABLE I Blood gases in investigated groups at rest during and after hypoxia

	Normal subjects			Cor pulmonale		
	At rest	During	1 h after	At rest	During	1 h after
Oxygen saturation of arterial blood (%)	97 9 ± 3 1	84 8 ± 4 9	97 8 ± 2 9	85 1 ± 6 8	68 7 ± 8 1	86 0 ± 7 1
Partial pressure of CO_2 in arterial blood (mm Hg)	–	–	–	41 1 ± 3 1	39 6 ± 3 0	40 9 ± 3 1
pH of arterial blood	7 37 ± 0 03	7 39 ± 0 04	7 36 ± 0 03	7 39 ± 0 03	7 41 ± 0 04	7 40 ± 0 04
Heamatocrit (%)	43 8 ± 4 1	43 5 ± 3 9	13 7 ± 4 0	46 9 ± 3 8	47 2 ± 4 5	47 9 ± 4 1

TABLE II Values of the course of ventricular contraction in investigated patients at rest and during hypoxia

Isometric contraction		Isotonic contraction		Total systole		Mechanical quotient	
At rest	Hypox	At rest	Hypox	At rest	Hypox	At rest	Hypox.
Healthy subjects (millisec)							
96 7 11 1	97 5 ± 10 8	270 4 ± 19 2	272 5 ± 17 6	367 1 ± 18 8	370 0 ± 19 1	2 87 ± 0 5	2 84 ± 0 4
Cor pulmonale (millisec)							
99 5 ± 10 2	108 5 ± 9 3	237 5 ± 17 7	239 0 ± 16 8	337 0 ± 17 1	347 5 ± 18 0	2 42 ± 0 2	2 23 ± 0 2

On the other hand in patients with chronic cor pulmonale statistically significant prolongation of the total isometric contraction and reduction of the mechanical quotient were observed The isotonic contraction did not change

3 Plasma sodium and plasma and erythrocyte potassium levels

In both examined groups we observed at the saturation minimum and for one hour after a slight decrease of average plasma sodium levels The variation of values is however very wide and this change is statistically insignificant ($p > 0.10$) (fig 1)

In both groups there was however, at the maximal hyposaturation a highly significant increase of the plasma potassium levels (on an average by 0 5 mEq/l — $p < 0.001$) This increase was observed in all examined subjects but one

tent At the end of the inhalation of this poor mixture" another specimen of arterial blood was withdrawn, the inhalation was stopped, and for another hour the patient inhaled atmospheric air After another hour further blood specimens were collected The subsequent samples were withdrawn on the next morning (24 hours after the beginning of the examination)

In the patients simultaneous recordings of ECG, phonocardiogram and sphygmogram of the carotid artery using Multiscriptor Hellige apparatus were made Using the method previously described (23) we calculated from this polygraphic record the main components of cardiac contraction total isometric and isotonic contraction and the mechanical quotient (ratio isotonic/isometric contraction) The recording was carried out in the patients before the examination at rest, at the maximal decrease of oxygen saturation and one hour after termination of the inhalation of poor mixture" always concurrently with the withdrawal of the arterial blood

The blood for the estimation of pH and blood gases was withdrawn strictly anaerobically The oxygen saturation of arterial blood was assessed by means of a flow cuvette with a Waters oximeter and manometrically with a Van Slyke apparatus (39) The partial carbon dioxide pressure was calculated from the manometrically assessed CO_2 content and the pH of arterial blood according to Van Slyke and Neill (39) and Milch et al (32) The pH of arterial blood was estimated on Cambridge and Godart pH meters at a temperature of 37 C The potassium and sodium concentrations of the specimens were estimated on a Zeiss flame photometer and the potassium concentration in red blood cells was assessed according to Lans et al (27)

In order to ascertain whether the observed changes were really the response to the investigated stimulus and were not distorted by a non specific reaction (e g variations in the electrolyte levels and excretion during 24 hour cycle) we subjected 7 healthy subjects and 7 patients with cor pulmonale to an examination identical as regards timing and procedure but omitting inhalation of the gas mixture with the low oxygen content The homogeneity of the response to the investigated stimulus was tested by means of Dixon s formulae (15) and the statistical significance of the results was evaluated using the paired Student s t test

Results

1 Changes in the blood gases and the pH of arterial blood

The initial saturation in patients with cor pulmonale was 85 1 % (table I) At the point of lowest saturation it had declined on an average to 68 7 %, i e by about 16 % In the control group of healthy subjects the average initial saturation was 97 9 %, declining to 84 8 % (i e by about 13 %) The rate of onset of hypoxaemia showed no major differences between individual patients from the two groups The partial CO_2 pressure in arterial blood was examined only in patients with cor pulmonale, in whom it declined slightly (by 1—2 5 mm Hg) as the saturation fell to its minimum The pH of arterial blood shifted with the deep fall in saturation in both groups of patients towards the alkaline side (by about 0 02) The difference is not statistically significant

In respect of all these parameters the values one hour after the inhalation of the poor oxygen mixture hardly differed from the initial values

2 Changes in the course of cardiac contraction

In the group of healthy subjects the course of cardiac contraction assessed by the polygraphic record hardly changed throughout the examination (table II)

subjects (fig 2) During both nights (before and after the experiment) it was approximately the same and it increased in daytime, in patients subjected to hyposaturation the daily maximum was later than in the control group but the difference was not statistically significant

In patients with cor pulmonale the curve of urinary sodium excretion was somewhat different the total amount of sodium excreted was smaller and the difference between the day and night period was less marked But here too there were no significant differences between the patients with and without inhalation of poor oxygen mixture

A similar position as regards the potassium excretion was found in healthy subjects (fig 3) Here too a maximum in daytime was apparent and the curves did not differ in the patients subjected or not subjected to hyposaturation

Some difference was observed in patients with cor pulmonale in the patients without inhalation of poor oxygen mixture the potassium excretion curve was flat with only a slight maximum in daytime and the total amount of potassium excreted was substantially lower than in healthy subjects In patients with cor pulmonale subjected to hyposaturation the potassium excretion more than doubled ($p < 0.001$) during the five hour period after the experiment and during the subsequent seven hour period it was still higher than in controls The potassium excretion during both night periods (before and after the examination) was approximately the same as in patients with cor pulmonale not subjected to hyposaturation

Discussion

In the patients with heart failure in different cardiopathies incl chronic cor pulmonale, a reduced potassium concentration in red blood cells and skeletal muscle has repeatedly been described as well as a rise of the plasma potassium levels In the absence of cardiac failure (as in our group of patients) two syndromes are present in chronic cor pulmonale which can especially in this disease influence the changes in electrolyte distribution the reduced oxygen saturation of arterial blood and hypercapnic acidosis

The effect of artificially produced hypercapnia and metabolic and respiratory acidosis on potassium and sodium metabolism was repeatedly investigated in the present experiments, which produced a satisfactory model of hypercapnic acidosis in man Less known is the mechanism of action in hypoxia Experiments assembled hitherto did not correspond exactly to the relatively slight hypoxaemia encountered in most patients with cor pulmonale Either there were only model experiments with human erythrocytes or else experiments on animals with excessive hypoxaemia as compared with conditions encountered in human pathology

We investigated therefore a short term and relatively slight hypoxaemia (reduction of saturation by 13—16 %) which is a quantitatively similar change to that observed in patients with chronic cor pulmonale e g during exacerbations of chronic bronchitis or in bronchopneumonia Our results suggest that even this slight degree of hypoxaemia

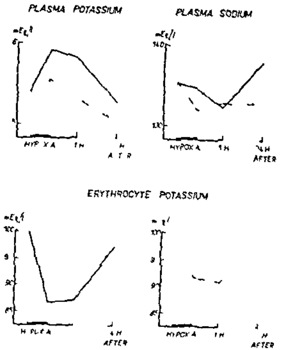


Fig 1 Changes in the potassium and sodium plasma levels and in the potassium concentration in red blood cells during and after short term hypoxaemia
— patients with cor pulmonale — — — normal subjects

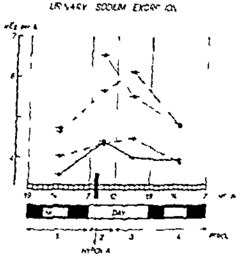


Fig 2 Average urinary sodium excretion during the diurnal cycle in examined patients
— cor pulmonale with hypoxia, — — cor pulmonale without hypoxia — — normal subjects with hypoxia, normal subjects without hypoxia

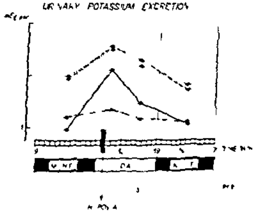


Fig 3 Average urinary potassium excretion during diurnal cycle in examined patients Symbols as in fig 2

One hour later the potassium plasma level began to decline somewhat and 24 hours after the experiment it practically reached initial values

The potassium content of red blood cells in both investigated groups at the saturation minimum decreased markedly and highly significantly (by 10—13 mEq/l, $p < 0.001$) likewise in all examined subjects but one This decrease was still apparent one hour after poor oxygen mixture inhalation Twenty four hours after the experiment the potassium content of red blood cells was the same as initially

In patients whose sodium and potassium values were assessed after the same time intervals but without inhalation of the poor oxygen mixture the values varied within the range of experimental error and their average did not change significantly

4 Urinary sodium and potassium excretions

In patients with or without inhalation of a poor oxygen mixture the urinary sodium excretion was very similar in character to that in the group of healthy

saturation of arterial blood was induced by inhalation of a gas mixture with a low oxygen content Values in the course of cardiac contraction, the plasma sodium plasma potassium and red cell levels were investigated as well as the urinary sodium and potassium excretions

In both examined groups already during hyposaturation there develops a marked increase of the plasma potassium levels and a reduction of the red cell potassium content The changes persist for one hour after the termination of hyposaturation and initial values are reached within 24 hours In patients with chronic cor pulmonale an increased urinary potassium excretion was observed during the period immediately after the induced hyposaturation In healthy subjects the potassium excretion does not differ from the normal diurnal cycle In neither of the two groups were changes found in the plasma sodium levels or the urinary sodium excretion

In patients with cor pulmonale, prolongation of isometric contraction during hypoxaemia as an index of the loss of contractility of myocardium was found In healthy subjects these changes did not occur

The mechanism of the described changes is discussed

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causes during the experiment a liberation of about 10 % of the potassium from erythrocytes and a rise of the plasma potassium levels It is interesting that in this respect we were unable to provide evidence of qualitative and quantitative changes in healthy subjects and in patients with chronic cor pulmonale

Changes in the sodium and potassium excretions had to be evaluated carefully in view of the variations in these values in the course of day and night We selected therefore a relatively long period for investigating these changes, and the results were always evaluated only in relation to control groups without inhalation of the "poor oxygen mixture" Our results indicate that in healthy subjects the daily potassium excretion curve does not change after short-term hypoxia, the change in the ratio of plasma potassium to erythrocyte potassium is quite reversible and the liberated potassium returns within 24 hours to the cells On the other hand, the increase of potassium excretion in patients with cor pulmonale immediately after hyposaturation shows that part of potassium liberated from the cells is probably lost by the organism through renal mechanisms For sodium we did not find in either of the examined groups significant changes in hypoxia, either in the plasma level or in the urinary excretion

We were able to confirm by the selection of patients and previous examination of blood gases and haemodynamic data, that in the examined subjects there was really only a hyposaturation of arterial blood as an experimental stimulus Influences of hypercapnia acidosis, cardiac failure, treatment with cardiotonics and diuretics, were eliminated in this way

In healthy subjects we did not observe any changes in the contractility of the heart muscle, investigated by the examination of polygraphic records In patients with cor pulmonale changes were found This difference between these two groups may be caused by a greater hypoxaemia in cor pulmonale the degree of hyposaturation was the same in the both groups (by 13 and 16 %) but the resulting saturation in patients with cor pulmonale was markedly lower than in healthy subjects (68 7 % and 84 8 %) The mechanism of the loss of contractility during hypoxaemia is not clear and cannot be ascribed only to a loss of the potassium from the cells, changes in the electrolytes in red blood cells are not the same as the changes in myocardial electrolytes There are other metabolic mechanisms who can play a role in the loss of contractility e g alteration of glycolysis ADP and ATP in the myocardium or the activation of the sympatho adrenal system

From our results it can be concluded that some changes in electrolyte levels and alterations in myocardial contractility in the patients with chronic cor pulmonale ascribed usually to a cardiac failure or hypercapnic acidosis, may be also explained as a result of hypoxaemia in these patients

Summary

In a group of 12 healthy subjects and 11 patients with chronic cor pulmonale a short term reduction in the oxygen

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A Procedure for in Vitro Studies on Fatty Acid Metabolism by Human Subcutaneous Adipose Tissue

By

JAN ÖSTMAN

During the last decade it has become evident that the free fatty acids (FFA) of plasma constitute a major source of substrate for the oxidative metabolism of peripheral tissues (15 23) The physiological significance of FFA and the mechanisms of their transport have been surveyed in detail by Fredrickson and Gordon (20), Borgstrom (7) Fritz (21) and Dole (17)

Various nutritional and endocrine factors are known to regulate the mobilization of FFA from adipose tissue (AT) Due to anatomical difficulties in obtaining blood samples from vessels exclusively draining adipose tissue, information on the mobilization of FFA has been gained mainly by determinations of the concentration and the *turnover* of the FFA in plasma An increased concentration of FFA in plasma is observed in man in e g , the fasting state (15 23) during stress (5 10), after surgical trauma (53) after exercise (2 11) and in diabetes mellitus (3, 32) After administration of *norepinephrine* (29), epinephrine (15, 23), growth hormone (18, 38) and some other hormonal factors the concentration of the FFA in plasma is increased in man Conversely the administration of glucose or insulin produces a sharp drop in normal man (15 23) and a more delayed fall in diabetic subjects (3) Reviews on the endocrine regulations of lipid mobilization have been published (19 30, 37, 44)

Most states and agents known to affect the mobilization of the FFA *in vivo* from adipose tissue produce the same effects *in vitro* when adipose tissue is incubated according to the methods first described by Reshef and Shapiro (41) and by Gordon and Cherkes (24) Thus adipose tissue from fasting (24, 41) and alloxan-diabetic rats (54, 55) is observed to release more FFA *in vitro* than tissues from fed and normal rats respectively Addition of norepinephrine (56), epinephrine (24 40) and several other hormonal factors to an albumin containing medium also produces an

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Biopsy procedure

All subjects were fasting overnight and not allowed to get out of bed before the biopsy procedure which started around 8 a m A teflon catheter was inserted into one brachial artery under local anesthesia Physiological saline solution without heparin was used to maintain patency of the catheter 30 minutes later a local anesthesia was produced by intradermal injections of 7—8 ml of a 1 % prilocaine solution[1] (Citanest Astra) in a rhomboidal figure (side 4 cm) on the upper lateral aspect of the thigh In the centre of this field a small intradermal prilocaine injection was necessary A 2—3 cm long incision was made 10 minutes later and a number of subcutaneous fat portions were dissected with minimal traumatization The total weight of fat tissue removed ranged from 1 to 3 g The fat portions were immediately placed in a flask containing 10 ml of a Krebs Henseleit bicarbonate buffer pH 7 4 containing 1 % of human albumin and kept at 37 C The wound was sutured within 10 minutes after the incision and covered with a minor bandage In most subjects only slight hematomas developed and except in one case the discomfort was small Blood samples were withdrawn 20 minutes after the catheterization into syringes containing EDTA Na_2 (1 36 g/100 ml of water) For each 10 ml of blood 1 ml of this EDTA solution was used The EDTA plasma thus obtained was used as incubation medium for a part of the adipose tissue At the same time and at ten minute intervals i e at the times of anesthesia incision and suturation respectively blood samples were withdrawn into heparinized syringes for determination of blood glucose plasma FFA and plasma glycerol The timing of the different steps in the biopsy procedure is shown in fig 1

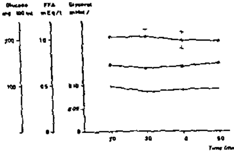


Fig 1 Arterial concentrations of blood glucose (●) plasma FFA (×) and plasma glycerol (○) in 4 non-diabetic and 13 diabetic subjects from whom subcutaneous fat portions were removed by a surgical procedure The catheter was inserted into a brachial artery at time 0 Anesthesia incision and suturation was performed at 30 40 and 50 minutes respectively Mean values ± S E

Incubation medium

A modified Krebs-Henseleit bicarbonate buffer was freshly prepared before each study from the following stock solutions 0 154 M NaCl (100 parts) 0 154 M $NaHCO_3$ (21 parts) 0 154 M KCl (4 parts) 0 154 M KH_2PO_4 (1 part) and 0 154 M $MgSO_4$ (1 part) Ca^{2+} was thus omitted from the originally described buffer solution (49) Bicarbonate solution was freshly prepared each week the other stock solutions at monthly intervals Lyophilized human albumin (Kabi AB Stockholm) was due to its high FFA content (1 20 mEq/M albumin) treated according to Goodman (22), by which method most of the FFA was removed Untreated and treated lyophilized albumin were then mixed in a ratio providing a final FFA content of 0 45 μEq/ml of the albumin solution used for the incubation (50 mg human albumin per ml KHA) The purity of the albumin preparation was checked by electrophoretic analysis in the Tiselius apparatus A 1 % solution of albumin in veronal buffert pH 8 6 and ionic strength (μ) 0 1 was run during 4 hours in a long cell at a potential gradient of approximately 6 volts/cm Only a single peak with the mobility of human albumin was observed during the experiment No insulin like activity of the albumin fraction was demonstrable when the $C^{14}O_2$ production by rat adipose tissue was studied according to Martin et al (36) with a modified procedure (13) in which a 5 % albumin solution was used By immuno-assay

[1] In separate experiments was ascertained that prilocaine *per se* did not influence the metabolic parameters studied

increased release of FFA from the adipose tissue of certain species. On the other hand the presence of glucose in the medium inhibits the release of FFA from normal (24, 40) as well as alloxan-diabetic rats (8). Endocrine effects on the fatty acid metabolism of adipose tissue *in vitro* are reviewed elsewhere (19, 37, 44, 57).

Most hormones known to increase the release of FFA *in vitro* also cause an increased release of glycerol, which indicates that a stimulation of the lipolysis is induced (33, 50). Evidence for an exaggerated lipolysis by administration of epinephrine has also been demonstrated by an increase in the di- and monoglycerides of rabbit adipose tissue (52). Features of a hormone-sensitive lipolytic enzyme have recently been described (4, 26, 43, 51). Glycerol once liberated from the tissue triglycerides is only insignificantly re-utilized within adipose tissue (45, 58). While the FFA formed by the lipolysis are to some extent re-esterified, glycerol takes no part in the triglyceride synthesis since it cannot be converted to alpha glycerophosphate, presumably due to the absence of glycerokinase in this tissue (34, 58). The glycerol release consequently reflects the degree of lipolysis better than does the release of FFA.

The uptake of FFA by adipose tissue has been measured by adding a tracer dose of radioactive FFA to the incubation medium (1, 45, 47). The FFA once taken up are under basal conditions rapidly esterified mainly to triglycerides, while only a smaller part remains unesterified or is oxidized (1, 47).

Studies *in vitro* on adipose tissue have most frequently been performed with the epididymal fat pad of the rat, due to the simplicity of that technique although adipose tissue from other localizations and species has been employed. The purpose of this investigation has been to develop a method for *in vitro* studies on human adipose tissue which hitherto has only been studied by few authors (12, 14, 28, 39). The method should allow determinations of the release and the uptake of FFA as well as of the release of glycerol and the uptake of glucose. It would then be possible to study the lipolytic and the esterification process in relation to the uptake of glucose. As the technique was intended to be applicable to out-patients without general anesthesia the methodology has been worked out for the use of subcutaneous adipose tissue. The metabolic parameters mentioned above have been studied during incubation in Krebs-Henseleit bicarbonate medium containing human albumin (KHA) as well as in plasma with the sodium salt of ethylenediaminetetraacetic acid present as an anticoagulant (EDTA plasma) from the subject studied. In a simplified form the technique has been used for a preliminary study of a small group of normal and diabetic subjects (12).

Material and methods

In the present investigation 4 healthy and 13 diabetic males were included. The diabetic patients were all new-discovered and the study was performed before treatment was started. Clinical and experimental data will be described in detail later (59).

extraction mixture of Dole and the volume made up to 10 ml with the same mixture Two aliquots of 3 ml each of the heptane phase obtained by addition of water and heptane to this extract and washed with 0 05 % H_2SO_4 were removed for titration of FFA After titration an equal amount of NaOH was added The heptane phase was transferred to counting vials with two heptane washings and NaOH was added when needed This pooled heptane extract is called the neutral lipid fraction After acidification with 0 2 ml of 0 05 % H_2SO_4 the FFA were extracted and transferred to counting vials by repeated heptane washings Radioactive palmitate and tripalmitin standards were simultaneously put through the same procedure in duplicate In table I is presented the radioactivity recovered in the neutral lipid and FFA fractions when palmitic acid 1 C¹⁴ and tripalmitin 1 C respectively were added to unlabeled tissue extracts In separate experiments glycerol was determined by the periodate and chromotropic acid reaction on aliquots of the aqueous phases from the tissue homogenates (31)

Standards Standards were run in each series of experiments

Glucose Glucose (Mallinckrodt Chem Co) dried over P_2O_5 was dissolved in $ZnSO_4$ + NaCl solution Aliquots sufficient for each series of determinations were put aside in the refrigerator and later diluted to a final concentration of 120 mg/100 ml ZnSO + NaCl solution

FFA Palmitic acid of reagent grade was purified by recrystallization Standard concentrations of palmitic acid in the heptane phase were 0 50 1 00 and 2 00 μEq/6 ml

Glycerol The concentration of glycerol (Merck) in reagent-grade samples was calculated from the specific gravity The standard concentration was 0 25 μ mole/ml in 6 % perchloric acid (w/v)

Palmitic acid l-C¹⁴ The specific activity of palmitic acid 1 C¹⁴ (Radiochemical Centre Amersham England) was 29 7 mc/mmole Separation of this batch according to the partition technique of Borgström (6) revealed

TABLE I Recoveries of labeled palmitic acid and tripalmitin in neutral lipid and FFA fractions when added to adipose tissue extracts

	No of exp	Recoveries[1] in	
		Neutral lipid fraction (%)	FFA fraction (%)
Palmitic acid	7	3 2±0 2	93 6±0 8
Tripalmitin	7	99 1±0 6	0 9±0 1

[1] Mean ± S E

that 96 1 % of the activity was recovered in the acid phase Before use the radioactive material was therefore purified according to the separation technique described in the section on Analytical methods (tissue lipids) The amount of activity used as standard was 0 10 μc

Tripalmitin I C¹⁴ The specific activity of tripalmitin 1 C¹⁴ (Radiochemical Centre Amersham England) was 25 1 mc/mmole No further purification of this batch was necessary since about 100 % of the radioactivity was found in the alkaline phase and since only an insignificant amount of activity was recovered in the acid fraction by the separation technique of Borgstrom (6) The amount of activity used as standard was 0 10 μc

Determination of radioa ti y

After addition of unlabeled palmitic acid as carrier the heptane phases were evaporated and counted twice for 10 minutes to an accuracy of at least 2 % in a Packard Tri Carb Liquid Scintillator Counter The solvent used was 10 ml of toluene containing 50 mg of 2 5-diphenyloxazole and 3 mg of 1 4 bis (4methyl 5phenyloxazolyle) benzene Quenching was corrected for by use of an internal standard

Calculations

The uptake of glucose and the release of FFA and glycerol were calculated as the difference in the concentrations of glucose FFA

according to Hales and Randle (27) no insulin was found in the albumin preparation Albumin (lot no TV Rdo 17) from the same batch was used for all incubations EDTA-plasma obtained during the biopsy procedure was immediately centrifuged for 20 minutes at 8,000 × g

After the addition of glucose and human albumin to the modified Krebs Henseleit bicarbonate solution, providing a final concentration of 1 mg glucose and 50 mg albumin per ml of the medium, the pH was adjusted to 7 4 with a few drops of 2 33 M NaOH Palmitic acid 1 C^{14} neutralized with 0 02 M NaOH in slight excess was dissolved at 35—40° C in a portion of the albumin solution By the addition of 0 06 μC of palmitic acid 1 C^{14} per ml of the albumin buffer a radioactivity of approximately 60 000 c p m per ml of the medium was obtained The same amount of labeled palmitate was added to the EDTA plasma By autoradiographic assay of the EDTA-plasma proteins separated by electrophoresis no radioactivity was detectable outside the albumin position The two incubation solutions were kept at 37° C for about one hour before the incubation

Preparation of adipose tissue

Shortly before the incubation was started about 45 minutes after the biopsy procedure, macroscopic connective tissue was carefully and quickly dissected from the adipose tissue The fat portions were rinsed in albumin buffer solutions and cut into 25—50 mg pieces The total wet weight of adipose tissue incubated in each medium ranged from 100 to 300 mg Approximately a third of the total adipose tissue excised was not incubated but divided into two parts and homogenized at the time when the incubation was started The wet weight of the incubated tissue was obtained by weighing the flask plus contents before and after the incubation

Incubation

The incubation started 45 minutes after the biopsy procedure with the fat portions distributed among two KHA media and two EDTA-plasma media, each 6 3 ml Simultaneously control media without tissue were incubated The incubation was performed during two hours at 37° C with air as gas phase and with gentle shaking (60 oscill /min) in a water bath

Analytical methods

Arterial blood Glucose was determined according to the enzymatic method of Marks (35) FFA was determined with the Dole procedure (16) as modified by Trout (48) on samples of heparinized plasma, extracted immediately after centrifugation Beta hydroxybutyric acid was observed not to interfere with the determination of FFA Glycerol was analyzed enzymatically according to Wieland (58) All determinations were run in duplicate

Incubation medium Glucose, FFA and glycerol in the medium were determined with essentially the same methods as used for the arterial blood

Glucose Two 0 2 ml samples from the tissue containing medium and four 0 2 ml samples from the control medium were used The volume of the aliquots from the protein free filtrate used for the final colorimetric reaction was generally 0 2 ml When the glucose concentration exceeded 200 mg/100 ml, only 0 1 ml was used

FFA Two 2 ml samples were removed from all media for determination according to Dole (16) as modified by Trout (48) Two aliquots of the final heptane phase were used for titration Two other aliquots were used for determination of the radioactivity of control medium after the incubation

Glycerol One 1 5 sample was removed and deproteinized with 0 3 ml of 30 % perchloric acid Two aliquots were used for the enzymatic determination Recovery of glycerol added to EDTA plasma was 100 %

Tissue lipids

At the start of the incubation two fat portions were used for determination of the initial FFA concentration in tissue The incubated fat portions were thoroughly rinsed in albumin and NaCl solutions before homogenization All tissues were extracted by all glass homogenization with 3 ml of the

extraction mixture of Dole and the volume made up to 10 ml with the same mixture Two aliquots of 3 ml each of the heptane phase obtained by addition of water and heptane to this extract and washed with 0 05 % H_2SO_4 were removed for titration of FFA After titration an equal amount of NaOH was added The heptane phase was transferred to counting vials with two heptane washings and NaOH was added when needed This pooled heptane extract is called the neutral lipid fraction After acidification with 0 2 ml of 0 05 % H_2SO_4 the FFA were extracted and transferred to counting vials by repeated heptane washings Radioactive palmitate and tripalmitin standards were simultaneously put through the same procedure in duplicate In table 1 is presented the radioactivity recovered in the neutral lipid and FFA fractions when palmitic acid 1 C¹⁴ and tripalmitin 1 C¹⁴ respectively were added to unlabeled tissue extracts In separate experiments glycerol was determined by the periodate and chromotropic acid reaction on aliquots of the aqueous phases from the tissue homogenates (31)

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Calculations

The uptake of glucose and the release of FFA and glycerol were calculated as the difference in the concentrations of glucose FFA

TABLE II Analytical errors of the different determinations made on the incubation medium and adipose tissue

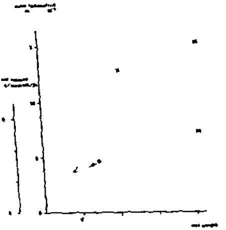
	No of duplicates	Range	Mean value	Analytical error	Analytical error %
Glucose mg/100 ml (medium)	81	76.0–286.1	151.1	1.8	1.2
FFA (medium) µEq/ml medium	53	0.485–1.102	0.655	0.005	0.8
FFA (tissue) µEq/ml homog. AT	74	0.020–0.560	0.131	0.008	6.1
Glycerol µmole/ml medium	58	0.016–0.192	0.076	0.004	5.3
Total tissue activity c.p.m./AT	25	1 830–54 840	12 500	350	2.8
Radioactivity of neutral tissue lipids c.p.m./AT	24	750–82 270	10 000	50	0.5
Radioactivity of tissue FFA fraction c.p.m./AT	24	700–4 640	2 250	90	4.0

TABLE III Individual changes of blood glucose, plasma FFA and plasma glycerol during the biopsy procedure. Mean individual changes (±S.E.) are calculated as the difference from the initial value 10 minutes before the anesthesia. Positive value indicates an increased concentration

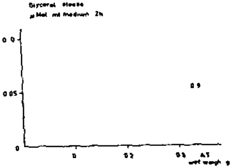
	No of subjects	At the time of		
		Anesthesia	Incision	Suturation
Glucose (mg/100 ml)	17	−0.07 ±1.61	−3.38 ±4.59	−[illegible] ±4.9[illegible]
FFA (mEq/l)	17	−0.005±0.009	−0.014±0.016	−0.016±0.018
Glycerol (mMole/l)	17	0.009±0.010	0.005±0.003	−0.009±0.010

and glycerol between the incubation medium with adipose tissue and the control medium without adipose tissue after two hours incubation. The analytical errors for the different methods used were calculated for all incubation experiments according to the formula $s = \sqrt{\frac{\sum d^2}{2n}}$ where d = the difference between the two duplicates and n = the number of duplicates. Table I summarizes the analytical errors of the determinations of glucose, FFA and glycerol in the medium as well as the tissue concentration of FFA and the radioactivity of the tissue. The wide range of the parameters studied is caused by the heterogeneity of the clinical material including normal as well as diabetic subjects.

The formula $\sqrt{\frac{\sum d^2}{2n}}$ was also used for the metabolic parameters studied where d = the difference between the duplicate incubations and n = the number of subjects. Statistical calculations were otherwise made as recommended by Snedecor (46).



F g 2 Relat onsh ps between the net release of FFA (●) or the uptake of palm t c acid 1 C^{14}) and the wet we ght of incubated subcutane ous ad pose tissue
Subcutaneous fat port ons removed during surgical operat on were incubated for two hours n 6 3 ml of KHA med um conta ning 50 mg/ml of human album n (FFA content 0 55 μ Eq/ml of the med um) 1 mg ml of glucose and palmit c ac d 1 C (approx mately 70 000 c p m /ml of the med um)
Regress on equat ons $Y = 0\,93\,x + 0\,175$
$Y_1 = 48\,7\ 10^4 x + 58$
Y net release of FFA n μ Eq med um
Y_1 = t ssue rad oactiv ty in c p m
x the wet we ght of ad pose tissue in g



F g 3 Relat onsh p between the release of glycerol and the wet weight of the incubated subcutaneous ad pose t ssue
Subcutaneous ad pose t ssue removed from a non-diabetic subject during surg cal operat on was incubated for two hours in 6 3 ml of KHA med um Regress on equat ons $Y = 0\,30x - 0\,010$
Y glycerol release in μ Mole/ml medium
x = the wet we ght of incubated adipose tissue in g

Results

Fig 1 shows the mean arterial con centrations of blood glucose plasma FFA and plasma glycerol during the biopsy procedure performed in the 4 non diabetic and 13 diabetic males Table III gives the corresponding in d vidual deviations when calculated as the difference from the initial values No significant alterations were observed in blood glucose plasma FFA or plasma glycerol

Relationships between the metabolic para meters studied and the wet weight of the incubated tissue

In some experiments a larger amount of subcutaneous adipose tissue was removed from non diabetic subjects during surgical operation and a number of tissue portions incubated A strong correlation was observed between the net release of FFA and the wet weight ($r = 0\,95$ $p < 0\,001$) as well as between the amount of labeled palmitic acid taken up by adipose tissue and the wet weight ($r = 0\,96$ $p < 0\,001$) (fig 2) The mean percentage of total tissue activity recovered in the neutral lipid fraction was 81 8 with a standard deviation of 2 9 which indicates that the degree of esterification was equal for all tissues In another experiment the release of glycerol was observed to be equally well related to the wet weight ($r = 0\,91$ $p < 0\,001$) (fig 3) The

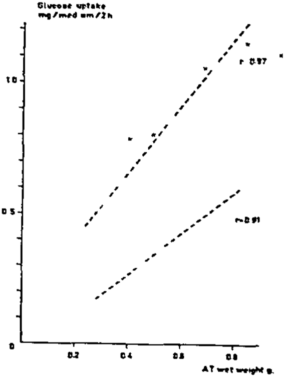


Fig 4 Relationship between the uptake of glucose and the wet weight of subcutaneous adipose tissue during incubation *in vitro*
Subcutaneous fat portions obtained from a non diabetic subject during surgical operation were incubated in 2 0 ml of KHA medium containing 20 mg/ml of human albumin and a glucose at a concentration of 1 0 mg ml or 2 5 mg ml Four tissue portions were first incubated for two hours in a medium of one particular glucose concentration and thereafter re-incubated for two hours in a medium containing the different glucose concentration
Regression equations $y_1 = 1.33x - 0.15$
$y_2 = 0.90x - 0.04$
y_1 and y_2 = disappearance of glucose in mg from medium with the initial glucose concentration of 2 5 mg ml (and 1 0 mg ml (● respectively
x = the wet weight of incubated adipose tissue in g

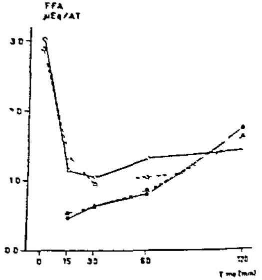


Fig 5 Changes in the FFA concentrations of subcutaneous (○) and omental (△) adipose tissue and of KHA medium (containing subcutaneous adipose tissue (●) and omental adipose tissue (▲)) during incubation *in vitro*
Samples of subcutaneous and omental adipose tissue were simultaneously removed during a surgical operation from a non-diabetic subject Incubations were performed in 5 0 ml of KHA medium containing 50 mg ml of human albumin (FFA content 0 45 µEq ml medium) for different length of time Each value represents the mean of duplicate incubations.

uptake of glucose into adipose tissue incubated in albumin media containing 1 0 mg and 2 5 mg glucose per ml respectively is shown in fig 4 All tissue sections were incubated twice in media of different glucose concentration The correlation between the uptake of glucose and the wet weight was apparent with the lower glucose concentration ($r = 0.91$ $p < 0.001$) as well as with the higher ($r = 0.97$ $p < 0.001$) Additionally it was shown that the uptake of glucose was higher from the medium with the greater glucose concentration being roughly twice as high as from the medium with the lower glucose concentration

Time course of the net release of FFA release of glycerol and uptake of FFA

A time course is presented for the amount of FFA released fig 5) and taken up (fig 6) by subcutaneous and omental adipose tissue obtained from a non diabetic subject during surgical operation The concentration of tissue FFA

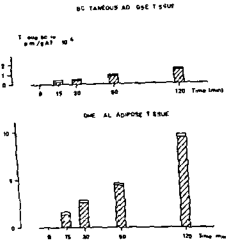


Fig 6 Uptake and esterification to neutral lipids of palmitic acid 1 C^{14} by subcutaneous and omental adipose tissue during incubation *in vitro*
The experimental conditions are the same as in fig 2 The initial activity in the KHA medium was approximately 65 000 c p m /ml
White bars = activity recovered as tissue FFA cross-hatched bars = activity recovered as neutral tissue lipids

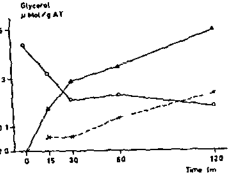


Fig 7 Changes in the glycerol concentrations of subcutaneous adipose tissue and KHA medium during incubation *in vitro*
Samples of subcutaneous adipose tissue removed during a surgical operation from a non-diabetic patient were incubated in 5 0 ml of KHA medium for different lengths of time Glycerol was determined by the periodate chromotropic acid method Each value represents the mean from duplicate incubations
○ = tissue glycerol ▲ = medium glycerol × = net produced glycerol in tissue + medium

initially the same for both types of tissue altered in a similar way during the incubation for both tissues After a rapid decrease during the first incubation period there was a slow increase during the subsequent incubations During these the net release of FFA was proportional to the time of incubation, while there was a higher net release of FFA during the first incubation period, presumably due to a release of pre existing tissue FFA While the net release of FFA was of the same magnitude for subcutaneous and omental tissue the uptake of labeled palmitate was markedly higher with omental fat, being higher by tenfold after two hours' incubation The percentage of total tissue activity recovered in the neutral lipid fraction at this time was also higher for omental fat (about 95 %) than for tissue of subcutaneous origin (about 65 %) The specific activity of the tissue FFA (in c p m per μEq FFA) was constant during the incubations, while the incorporation of labeled palmitate into the neutral tissue lipids proceeded proportionally to the time of incubation

A release of pre existing tissue glycerol also appeared to occur initially during the incubation (fig 7) but beyond 30 minutes the release of glycerol proceeded linearly

Effect of EDTA on the lipolysis and the esterification

In table IV are shown the results from different experiments when EDTA Na_2 was added to the KHA medium in the same concentration as used in the plasma medium No significant effect was ob

TABLE IV. Effects of EDTA Na$_2$ (3.7×10^{-4} M) on the glucose uptake and the fatty acid metabolism of human adipose tissue *in vitro*. Mean ± S.E.

Medium	Exp. A[1] Glucose uptake mg/g AT/2 h	Exp. B[2] FFA net release μEq/g AT/2 h	FFA tissue content μEq/g AT
KHA	0.45±0.05	1.34±0.20	1.75±0.11
KHA+EDTA	0.47±0.03	1.44±0.29	1.44±0.17

		Exp. D[3] Radioactivity recovered in	
	Exp. C[2] Glycerol release μMole/g AT/2 h	Neutral tissue lipids c.p.m./g AT	Tissue FFA c.p.m./g AT
KHA	0.18±0.01	136,900±11,900	18,900±1,100
KHA+EDTA	0.23±0.03	143,100±10,400	16,280±1,020

[1] Incubation medium: 2.0 ml of KHA containing 20 mg/ml of human albumin and 2.0 mg/ml of glucose. n = 7.

[2] Incubation medium: 3.0 ml of KHA containing 50 mg/ml of human albumin (FFA content 0.55 μEq/ml medium). n = 6.

[3] Incubation medium: 6.3 ml of KHA containing 20 mg/ml of human albumin, 1.0 mg/ml of glucose and palmitic acid-1-C^{14} (approximately 65,000 c.p.m./ml medium). n = 4.

served on the net release of FFA and glycerol or on the tissue FFA concentration. Additionally it was shown that EDTA did not influence the uptake of FFA, the incorporation into the neutral lipid fraction or the uptake of glucose.

Studies on the release of radioactivity once taken up by adipose tissue

Tissues once incubated for one hour in labeled KHA medium were in one set of experiments re-incubated in unlabeled medium. The results from a typical experiment are given in table V. These subcutaneous fat sections were obtained from a diabetic patient and re-incubation was performed in EDTA-plasma from the subject. While a certain amount of the tissue activity was released during the re-incubations in EDTA plasma only insignificant activity was recovered as neutral lipids in the medium. The concentrations of labeled neutral tissue lipids and of tissue FFA were similar for all tissue samples, whether re-incubated or not. During re-incubation, however, the activity recovered as tissue FFA decreased rapidly, although a certain amount was still found after two hours incubation.

Comparisons with respect to the metabolic parameters between two tissue samples incubated under identical conditions and obtained from the same subject

In table VI is presented the range of the various metabolic parameters studied

TABLE V Distribution of radioactivity in human subcutaneous adipose tissue and radioactivity released when transferred to unlabeled medium after initial incubation in albumin medium containing palmitic acid 1 C^{14}

Subcutaneous fat portions removed during local anesthesia from a diabetic subject were all prelabeled during one hour in 20 ml of KHA medium containing 50 mg/ml of human albumin (FFA content 1 04 µEq/ml medium) 3 0 mg/ml glucose and palmitic acid 1 C^{14} (specific activity of medium FFA 145 000 c p m./µEq) The tissues were thoroughly washed in albumin and NaCl solutions whereafter four samples (3—6) were each transferred to 5 ml of EDTA plasma and incubated for 15 or 120 minutes

Tissue section	Time of re incubation (min)	Activity recovered in tissue: Neutral lipids c p m./g AT	FFA c p m /g AT	Tissue FFA conc µEq/g AT	Activity recovered in medium: Neutral lipids c p m./g AT	FFA c p m./g AT
1	0	28 200	17,200	1 27	—	—
2	0	28 900	20 600	0 95	—	—
3	15	22 600	2 800	1 16	100	3 900
4	15	27 000	4 000	1 46	<100	7 600
5	120	29 100	1 500	1 09	<100	13 000
6	120	33 600	1 200	1 45	<100	10 600

TABLE VI Comparison between KHA and EDTA plasma in respect of the metabolic parameters in duplicate incubations

	Medium	No of dupl	Mean value	Range	Range of difference	Variation ±	Variation
Glucose uptake mg/g AT/2 h	KHA	16	1 86	0 27 – 4 58	0 08 – 1 85	0 64	34 4
	EDTA plasma	16	2 93	0 13 – 11 93	0 06 – 3 87	0 99	33 8
FFA net release µEq/ml medium/g AT/2 h	KHA	17	0 374	0 047 – 0 975	0 002 – 0 200	0 085	22 7
	EDTA plasma	17	0 376	0 076 – 0 830	0 005 – 0 191	0 031	21 5
Glycerol release µmole/ml medium/g AT/2 h	KHA	16	0 223	0 046 – 0 558	0 – 0 102	0 042	18 8
	EDTA plasma	15	0 183	0 040 – 0 469	0 013 – 0 123	0 036	19 7
Total tissue activity c p m/g AT	KHA	14	3 330	18 610 – 263 900	40 – 24 770	8 800	12 0
	EDTA plasma	14	58 110	14 410 – 310 670	670 – 23 760	6 900	11 9
Radioactivity in the neutral tissue lipids in % of total tissue activity	KHA	14	70 2	14 1 – 97 8	0 6 – 10 1	3 8	5 4
	EDTA plasma	14	62 8	12 9 – 9 9	0 – 12 1	4 0	6 4

during incubation in KHA medium and EDTA-plasma, i. e. the uptake of glucose, the release of FFA and glycerol and the uptake of labeled palmitic acid into adipose tissue. The incorporation of palmitic acid-1-C^{14} into the neutral tissue lipids is given in per cent of total tissue activity. The metabolic activity of adipose tissue was of the same magnitude whether incubations were performed in KHA medium or EDTA-plasma. In the same table are also shown the differences in the metabolic parameters observed with duplicate incubations. The variation of duplicate incubations was estimated as described earlier in the section on Analytical methods (calculations). The greatest variation, expressed in per cent, was observed for the uptake of glucose (about 30 %), while there was a small variation in the esterification capacity between two tissues from the same subject (about 6 %).

Discussion

An attempt has been made to use small pieces of human subcutaneous fat portions excised during local anesthesia for studies *in vitro* on the fatty acid metabolism and the uptake of glucose. Since fear, anxiety and other stress stimuli are known to affect the glucose and fatty acid metabolism, recognizable by an increased concentration of glucose (42) and plasma FFA (5, 10), it was of importance to follow the arterial levels of glucose, FFA and glycerol during the biopsy procedure. No evidence for a hyperglycemic reaction or an increased lipid mobilization was observed in these experiments.

In accordance with earlier reports on rat adipose tissue a diffusion of the pre-existing FFA and glycerol (23) of the tissue took place initially during the incubation. Thereafter the net release of FFA and glycerol occurred at a quite constant rate, so that the release of FFA and of glycerol could be used as measures of the lipolytic activity of the tissue, a pre-incubation time of 45 minutes was chosen.

The uptake of glucose, the release of FFA and glycerol and the uptake of labeled palmitate were all well related to the wet weight of the incubated tissue sample. These parameters can therefore be related to the wet weight in order to make comparisons between the different incubations possible.

The ratio of the activity incorporated into the neutral tissue lipids to the total tissue radioactivity was equal for adipose tissue of the same origin when incubated under identical conditions. This ratio might therefore offer a certain estimate of the esterification capacity and make comparisons possible when the fat portions are incubated under identical conditions.

In order to study the metabolic activity of adipose tissue in a more physiological milieu, incubations were performed also in plasma from the subject studied. Since it has been shown that EDTA inhibits the activity of a lipase (pH optimum 6.5—7.0) (4) of rat adipose tissue, the use of EDTA as an anticoagulant was not accepted until it was established that EDTA did not influence the release of FFA and glycerol. Additionally it was shown that EDTA did not affect the incorporation

of active palmitic acid into the neutral tissue lipids.

The net release of FFA and glycerol as well as the net uptake of FFA were observed to be much smaller per unit wet weight of human adipose tissue than has been described previously for rat adipose tissue under similar conditions of incubation. A considerably higher activity was observed in human omental adipose tissue than in subcutaneous adipose tissue in accord with a previous report (28). A higher uptake of glucose was generally demonstrated when subcutaneous adipose tissue was incubated in EDTA plasma as compared with KHA. This may be explained by the predominance of diabetic subjects included in this study since there is a higher uptake of glucose from plasma of diabetic subjects (12), at least partly due to a higher concentration of glucose. The net release of FFA and glycerol as well as the net uptake were on the other hand of the same magnitude whether subcutaneous adipose tissue was incubated in KHA or in EDTA plasma.

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Even if the metabolism *in vitro* of subcutaneous adipose tissue should differ at least in quantitative respects from that of fat depots at other sites, the methodology described will allow informative studies on the metabolism of human adipose tissue *in vitro* since the subcutaneous adipose tissue generally constitutes a great part of the total body fat.

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An incubation procedure adapted for isolated human adipose tissue is de-

scribed for studies on the glucose uptake, *the lipolysis and the esterification of FFA to triglycerides* During incubation *in Krebs Henseleit-bicarbonate-buffered* medium containing human albumin, *glucose and C^{14}-labeled* palmitate, simultaneous measurements were made *of 1) the disappearance of glucose from the medium, 2) the net changes of FFA and glycerol* in the medium, and 3) the distribution of radioactivity in the neutral tissue lipids and the tissue FFA

For incubation of fat tissue samples in plasma, the use of EDTA Na_2 as an anticoagulant was shown to be without detriment, since this agent was demonstrated not to influence either the lipolysis or the esterification process

The incubation technique worked out allowed, with satisfactory accuracy, measurements of the glucose uptake and the fatty acid metabolism by small subcutaneous fat samples removable during local anesthesia The biopsy procedure by which the fat portions were excised from non-diabetic and diabetic subjects did not alter the arterial concentrations of blood glucose, plasma FFA or plasma glycerol

The various metabolic parameters studied were all well related to the wet weight of the incubated tissue When using the net release of FFA and glycerol into the medium as measures of the lipolytic activity of the tissue, a certain time of pre-incubation was necessary Some FFA taken up by adipose tissue was esterified to triglycerides The ratio of the activity incorporated into the neutral tissue lipids to the total tissue radioactivity was equal for sections of the same origin when incubated under identical conditions, and therefore was used as a measure of the esterification capacity of the tissue By re incubation experiments it was shown that a significant part of the labeled palmitate once taken up by human adipose tissue was again released as FFA into the medium, while no release of neutral lipids was demonstrable

The release of FFA was of the same magnitude from subcutaneous and omental adipose tissue, while the uptake and the esterification occurred considerably more slowly in fat tissue of subcutaneous origin

Acknowledgements

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Studies on the Nitrogen Balance in the Human during Long-term Treatment with Different Anabolic Agents under Strictly Standardized Conditions

By

A Saarne L Bjerstaf and B Ekman

The ever increasing interest in anabolic steroids and their clinical application have induced us to carry out comparative examinations on the nitrogen retaining effect of a number of these anabolic steroids These examinations have in some cases been performed on the same patient under varying supplies of calories and protein A total of 4 patients have been observed over periods ranging between 42 and 347 days

The following anabolic steroids were involved in this examination Androxan® (Serono Rome, represented by Leo Ltd Halsingborg), Dianabol® (Ciba Products Ltd Stockholm), Deca Durabolin® (Organon represented by Pharmacia Ltd Uppsala) Anadur® (Leo Ltd Halsingborg) Primobolan® Depot Schering Ltd Saltsjo-Duvnäs)

Among the preparations included in our investigations, Anadur and Androxan are new products

Submitted for publication July 15 1964

Anadur, 19 nortestosterone p-hexoxyphenyl propionate is a parenteral, long acting anabolic steroid (4,10)

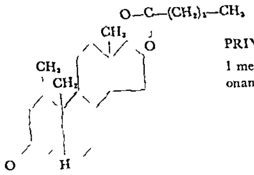
Androxan, 17β hydroxy 17α methyl androstan (3,2 C) isoxazole is an oral anabolic steroid (2, 5 10)

Androxan is closely related, chemically to androstanazole (Stromba Winthrop Ltd) (3)

Methods

The 24 hour basal metabolic rate for each patient was calculated according to Fleisch (6) before the experimental work was started

During the period under which the patients were under observation they were all fed by tube The diet fed was prepared from milk to which different amounts of glucose Meritene (The Dietene Company Minneapolis Minn U S A represented by Cortec Ltd Malmö) and Intralipid (Vitrum Stockholm) had been added so that the



PRIMOBOLAN DEPOT

1 methyl 1' androsten 17β-ol 3-on 17-onanthate

percentages of the proteins fats and carbohydrates virtually corresponded to the diet proposed by B Isakson (7)

Meritene is a protein vitamin mineral preparation 100 g of Meritene contain proteins 33 g (under consideration of all essential aminoacids) carbohydrates 58 4 g, vitamin A 7000 I U aneurin hydrochloride (B_1) 2 4 mg riboflavin (B_2) 4 3 mg nicotinic acid 22 mg pyridoxine (B) 330 mcg pantothenic acid 3 4 mg vitamin B_{12} 2 mcg ascorbic acid (C) 80 mg vitamin D 550 I U Ca 1 g P 0 8 g S 175 mg Mg 95 mg Na 0 5 g K 1 4 g Fe 15 mg Cu 0 11 mg I 0 09 mg

Intralipid is composed of fractionated soy bean oil 20 g fractionated lecithin from egg yolk 1 2 g glycerol 2 5 g made up to 100 ml with sterilized water

The dietary content of nitrogen was controlled once a week

Nitrogen was estimated according to Kjeldahl s method The urinary nitrogen output was determined from 24-hour urine portions The stools too were collected and the faecal nitrogen was estimated once or twice weekly

Both during the periods of observation and of treatment haemoglobin non protein nitrogen bilirubin alkaline phosphatase thymol flocculation (MacLagan s method) and oxalic acetate and pyruvate transaminases (SGOT and SGPT) were controlled every second week in cases 1 and 2 and every week in cases 3 and 4

The curves demonstrating the consecutive alterations in the nitrogen balance show the daily average net nitrogen gain or loss over a period of 7 days

The weights of the patients were controlled once a week

Case reports

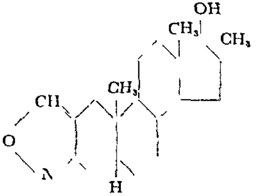
Case 1 Male aged 57 Diagnosis Huntington s chorea Severe dementia The patient was confined to bed and had previously temporarily been fed by tube

Weight 52 kg height 173 cm calculated basal metabolic rate 1356 calories The patient was fed by tube three times a day throughout the trial

During the first period of the study the patient received 1754 calories a day and the dietary caloric uptake surpassed the computed basal metabolic requirement by 29 % 70 g of protein i e 1 34 g/kg body weight were contained in the daily diet The protein proportion present in the total daily caloric supply was 16 % the proportion of fat was 36 % and that of carbohydrates was 48 %

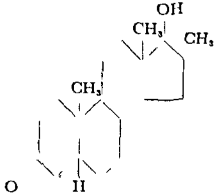
After a relatively short period of adaptation the patient received 50 mg of Anadur By mistake the injection was given subcutaneously The poor absorption by the subcutaneous tissue of steroids and a simultaneously occurring intestinal infection resulting in considerable losses in fluid produced a marked reduction of the patient s weight as well as the development of a negative nitrogen balance during the first week During the following two weeks (figs 1 A and 2 A) the nitrogen balance just barely reached a positive value

The chemical configuration of the steroids investigated appears from the following formulas



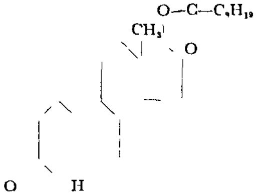
ANDROXAN

17β-hydroxy-17α methyl androstan-(3,2C) -isoxazole



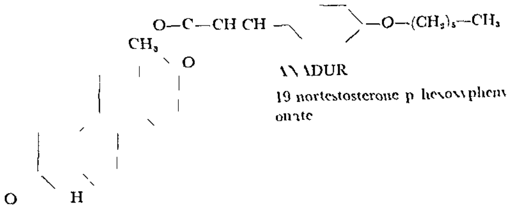
DIANABOL

17β-hydroxy-17α methyl androsta-1,4 dien -3-one



DECA DURABOLIN

19-nortestosterone-decanoate



ANADUR

19 nortestosterone p hexoxyphenyl-propionate

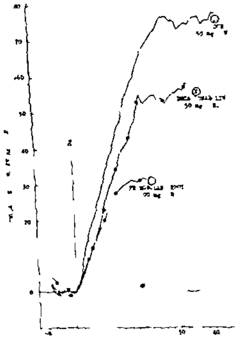


Fig 4 Case 1 Cumulative nitrogen balance during the second period of examination on subsequent oral and parenteral anabolic steroids

Intramuscular injection of 50 mg of Anadur led (figs 1 B and 2 B) in the following 28 days to a positive nitrogen balance which totalled 49 0 g

Following the oral administration of Androxan at a daily dosage of 10 mg (figs 1 C and 3 C) for 7 days and at a daily dosage of 15 mg (figs 1 D and 3 D) over a further period of 8 days total nitrogen retentions of 6 6 g and 5 9 g respectively were recorded

The intramuscular administration of 25 mg of Deca Durabolin (figs 1 E and 2 E) resulted in a positive total nitrogen balance of 18 3 g over a period of 18 days while 50 mg intramuscularly of Deca Durabolin produced a positive nitrogen balance totalling 30 9 g over a 20 day period (figs 1 F and 2 F)

Dianabol was given orally in daily doses of 15 mg during 14 days (figs 1 G and 3 G and produced a total nitrogen retention of 10 2 g

During the second period of this study the daily caloric supply was increased to 2 170 calories Thus the computed basal metabolic requirement was exceeded by 55 % The daily ration contained 106 g of protein i e 1 65 g/kg body weight The proportion of protein in the caloric supply was 19 5 % the proportion of fat was 35 % and that of the carbohydrates amounted to 45 5 %

Following a 3 week period of adaptation (fig 1 H) 50 mg of Anadur were given intramuscularly The nitrogen balance then turned strongly positive for 28 days and the cumulative nitrogen retention amounted to 76 1 g (figs 1 I and 4 I)

Androxan given orally in a daily dosage of 15 mg (figs 1 J and 3 J) over a period of 14 days produced a total nitrogen retention amounting to 22 1 g

Deca Durabolin 50 mg administered intramuscularly (figs 1 K and 4 K) resulted in a positive nitrogen balance during a period of 20 days the cumulative nitrogen retention being 54 7 g

A few days after the patient had received 100 mg of Primobolan Depot by intramuscular route (fig 1 L) infection of the urinary tract (fig 1 M) developed which led to a considerable pyrexia The current examination was interrupted by this condition

After the patient had recovered 100 mg of Primobolan Depot were given intramuscularly once more (figs 1 N and 4 N) This time the injection resulted in a 16 day positive nitrogen balance with a total nitrogen retention of 30 8 g

Case 2 Male aged 56 Diagnosis schizophrenia Owing to an oesophageal stricture resulting from an alkaline burn Witzel's gastrostomy had been performed 17 years ago and the patient was still being fed through *the tube He* was mostly confined to bed

Weight 66 5 kg height 176 cm calculated basal *metabolic rate* 1527 calories The patient was fed by tube three times a day The diet provided 1986 calories thus exceed*ing by* 7 5 % the computed caloric requirement The diet fed contained 79 g of protein i e 1 18 g/kg body weight The proportion *in the* caloric supply of protein was 15 9 % of

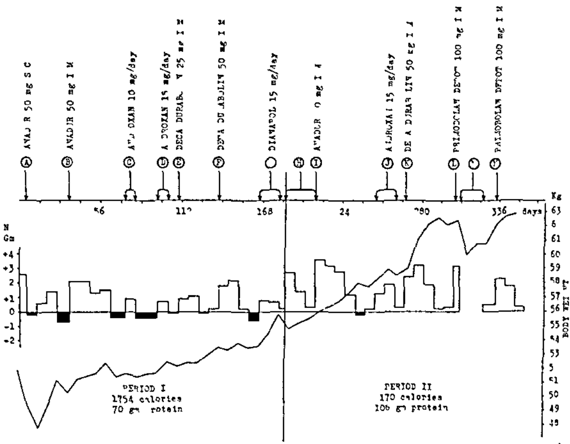


Fig 1 Case 1 Consecutive alterations in nitrogen balance and body weight subsequent to the administration of parenteral and oral anabolic steroids

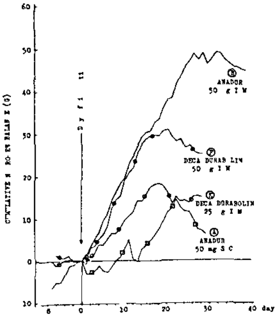


Fig 2 Case 1 Cumulative nitrogen balance during the first period of examination subsequent to the administration of parenteral anabolic steroids

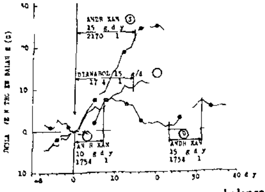


Fig 3 Case 1 Cumulative nitrogen balance during the first and second period of examination subsequent to the administration of oral anabolic steroids

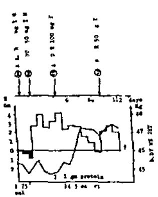


Fig 7 Case 3 Consecutive alterations in nitrogen balance and body weight subsequent to repeated administration of Anadur

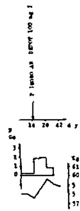


Fig 9 Case 4 Consecutive alterations in nitrogen balance and body weight subsequent to the administration of Primobolan Depot

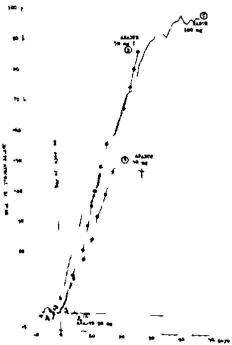


Fig 8 Case 3 Cumulative nitrogen balance subsequent to repeated administration of Anadur

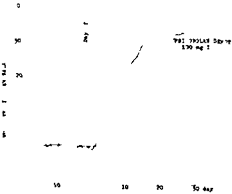


Fig 10 Case 4 Cumulative nitrogen balance subsequent to the administration of Primobolan Depot

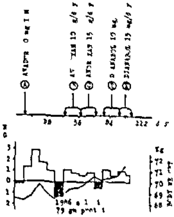


Fig. 5. Case 2. Consecutive alterations in nitrogen balance and body weight subsequent to the administration of parenteral and oral anabolic steroids.

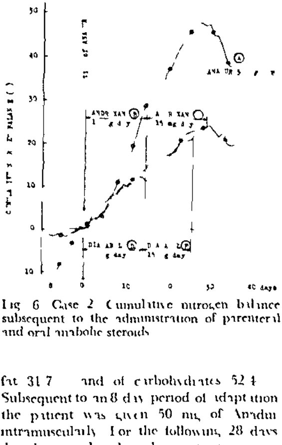


Fig. 6. Case 2. Cumulative nitrogen balance subsequent to the administration of parenteral and oral anabolic steroids.

fat 31.7 % and of carbohydrates 52.4 %. Subsequent to an 8 day period of adaptation the patient was given 50 mg of Anadur intramuscularly. For the following 28 days this dose produced a change to a positive nitrogen balance with a total nitrogen retention amounting to 18.1 g (figs. 5 A and 6 A).

Androxan in daily oral doses of 10 mg over a period of 14 days was followed by a total nitrogen retention of 13.6 g (figs. 5 B and 6 B). During the next 2 weeks the dosage was raised to 15 mg of Androxan daily (figs. 5 C and 6 C). *During this period the total* nitrogen retention was 10.3 g.

Daily doses of 10 mg of Dianabol orally over a period of 2 weeks (figs. 5 D and 6 D) produced a nitrogen retention totalling 11.4 g. When, during the following 11 days, the dosage was increased to 15 mg (figs. 5 E and 6 E), the nitrogen retention recorded was 9.6 g. At this point of time the patient was dismissed from hospital, so the study was discontinued.

Case 3. Female aged 75 who had undergone osteosynthesis for a fracture of the neck of the femure. About 4 weeks after the operation the patient began to refuse her food, thus necessitating continuous tube feeding.

Weight 45.3 kg, height 164 cm, calculated basal metabolic rate 1117 calories. *This patient was confined to bed throughout* the whole period of examination during which *she showed very poor motor activity.* At the beginning the patient received 1175 calories daily. The diet fed contained 78.1 g of protein, i.e., 1.7 g/kg body weight. The total caloric supply involved 26.5 %, protein 28.5 % fat, and 45 % carbohydrates.

During a 5 day period of adaptation the nitrogen balance was negative with an average of 0.1 g daily. After intramuscular administration of 50 mg of Anadur (fig. 7 A) the nitrogen balance remained negative throughout the following 7 days and the patient simultaneously lost 0.6 kg in weight. It was *evident from these observations that the* actual caloric supply which had been adjusted *to the computed basal metabolic requirements* was not sufficient for obtaining a positive nitrogen balance *despite the relatively* high supply in protein per kg body weight. We therefore, raised the daily caloric supply by 33 % to 1485 calories.

In this new high caloric diet the proportion of protein remained unaltered with 78.1 g or 1.7 g/kg body weight. In the total caloric supply there was 21.1 % protein 36.2 %, fat and 42.7 % carbohydrates.

After a 4 day period of adaptation to the raised caloric supply a further injection of 50 mg of Anadur was given intramuscularly

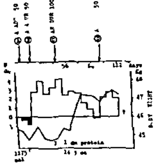


Fig 7 Case 3 *Consecutive alterations in nitrogen* balance and body weight subsequent to repeated administration of Anadur

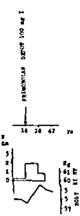


Fig 9 Case 4 *Consecutive alterations in nitrogen* balance and body weight subsequent to the administration of Primobolan Depot

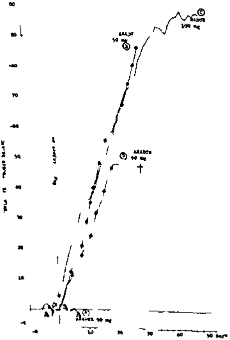


Fig 8 Case 3 Cumulative nitrogen balance subsequent to repeated administration of Anadur

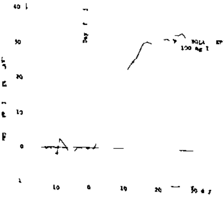


Fig 10 Case 4 Cumulative *nitrogen* balance subsequent to the administration of Primobolan Depot

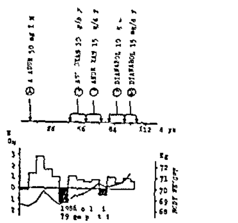


Fig 5 Case 2 Consecutive alterations in nitrogen balance and body weight subsequent to the administration of parenteral and oral anabolic steroids

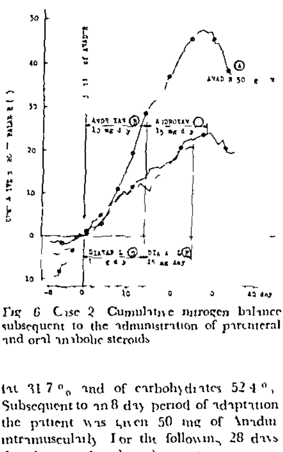


Fig 6 Case 2 Cumulative nitrogen balance subsequent to the administration of parenteral and oral anabolic steroids

fat 31 7 % and of carbohydrates 52 4 %, Subsequent to an 8 day period of adaptation the patient was given 50 mg of Anadur intramuscularly For the following 28 days this dose produced a change to a positive nitrogen balance with a total nitrogen retention amounting to 18 1 g (figs 5 A and 6 A)

Androxan in daily oral doses of 10 mg over a period of 14 days was followed by a total nitrogen retention of 13 6 g (figs 5 B and 6 B) During the next 2 weeks the dosage was raised to 15 mg of Androxan daily (figs C and 6 C) During this period the total nitrogen retention was 10 3 g

Daily doses of 10 mg of Dianabol orally over a period of 2 weeks (figs 5 D and 6 D) produced a nitrogen retention totalling 11 g When, during the following 11 days, the dosage was increased to 15 mg (figs 5 E and 6 E), the nitrogen retention recorded was 9 6 g At this point of time the patient was dismissed from hospital, so the study was discontinued

Case 3 Female aged 75 who had undergone osteosynthesis for a fracture of the neck of the femure About 4 weeks after the operation the patient began to refuse her food thus necessitating continuous tube feeding

Weight 45 3 kg, height 164 cm, calculated basal metabolic rate 1117 calories This patient was confined to bed throughout the whole period of examination during which she showed very poor motor activity At the beginning the patient received 1175 calories daily The diet fed contained 78 1 g of protein, i e , 1 7 g/kg body weight The total caloric supply involved 26 5 % protein 28 5 % fat, and 45 % carbohydrates

During a 5 day period of adaptation the nitrogen balance was negative with an average of 0 1 g daily After intramuscular administration of 50 mg of Anadur (fig 7 A) the nitrogen balance remained negative throughout the following 7 days and the patient simultaneously lost 0 6 kg in weight It was evident from these observations that the actual caloric supply which had been adjusted to the computed basal metabolic requirements was not sufficient for obtaining a positive nitrogen balance despite the relatively high supply in protein per kg body weight We, therefore raised the daily caloric supply by 53 % to 1485 calories

In this new high-caloric diet the proportion of protein remained unaltered with 78 1 g or 1 7 g/kg body weight In the total caloric supply there was 21 1 % protein 36 2 % fat and 42 7 % carbohydrates

After a 4 day period of adaptation to the raised caloric supply a further injection of 50 mg of Anadur was given intramuscularly

lower than 800 to 900 calories (7) Studies on the protein metabolism, employing N^{15} glycine and RISA (Radio iodine human serum albumin), have demonstrated that the protein metabolism is not influenced indirectly by anabolic steroids through inhibition of protein catabolism but that there is a direct influence, which expresses itself in an acceleration of protein synthesis (12) These studies have also shown the extent of protein synthesis to be exclusively dependent on the degree of the caloric and protein supply

All of our patients were fed by tube Thus the intake of the previously computed amounts of protein and calories was absolutely reliable By variation of the dietary protein and caloric contents we were also able to demonstrate the close relationship existing between the amount of the supply of protein and calories and the nitrogen retaining effect of anabolic steroids

In cases 1 (first period) and 2 to whom the daily protein and caloric supply had been kept at equal levels throughout the period of examination 50 mg of Anadur each time produced the same mean daily nitrogen retention (table I Nos 1 and 11) When the protein and caloric supply was higher as in case 1 (second period) and in case 3 there was a considerable increase in the *nitrogen retaining* effect of Anadur which was almost equal in both cases (table I Nos 7 and 16)

The importance of an increase in the protein supply for the nitrogen balance in one and the same patient is demonstrated by the results obtained in case 1 first and second periods) While the non protein nutritional factors were kept unchanged, the patient in the second period of the trial was given 51 % more protein than he had received in the first period The nitrogen retention produced by Anadur in the second period was shown to exceed that of the first period by 58 % Therefore an increase in the protein supply (51 %) resulted in an almost equal rise in the nitrogen retaining effect (58 %) (table I, Nos 1 and 7)

When the caloric and the protein supplies were kept at constant levels (case 1, first period), 50 mg of Deca Durabolin and 50 mg of Anadur produced approximately equal nitrogen retaining effects calculated per 24 hours (table I, Nos 1 and 5) The difference between the respective total nitrogen retaining effects of both drugs during a period of positive nitrogen balance was due to the prolonged duration of the effect of Anadur The effect of a 25 mg dose of Deca Durabolin was found to be considerably weaker than both 50 mg of Anadur and 50 mg of Deca Durabolin (table I, Nos 1 4 and 5)

An increase in the caloric and protein supply to the same patient (case 1 first and second *periods*) *produced* a rise in the nitrogen retaining *action of 50 mg* both of Deca Durabolin *and of* Anadur by 60 and 58 % respectively Thus both preparations showed equal *increases in* their activity (table I Nos 7 and 9) *Androxan* at a daily dosage of 15 mg likewise developed a considerably higher nitrogen retaining effect when the caloric and protein supply was raised (table I Nos 3 and 8)

One might of course assume that an increase of the caloric and protein supply

(figs. 7 B and 8 B). A positive nitrogen balance ensued during the following 4 weeks, totalling 86.1 g. After this period the patient was given 100 mg of Anadur intramuscularly (figs. 7 C and 8 C). This dose was followed by a positive nitrogen balance over a further period of 6 weeks, the total nitrogen retention amounting to 97.3 g.

After a 7 day control period, the patient was once again given 50 mg of Anadur intramuscularly (figs. 7 D and 8 D). In the course of the following 3 weeks her total nitrogen retention was 17.7 g. When, at the beginning of the 4th week, pyrexia and widespread bronchopneumonic infection developed, it was no longer possible to continue the examination.

One week later the patient died. Post mortem examination revealed the presence of cerebral atrophy, as well as widespread bronchopneumonia and severe generalized arteriosclerosis.

Case 4. Female aged 82. Diagnosis: oesophageal carcinoma. Witzel's gastrotomy had been performed because of complete oesophageal stenosis. Most of the time, the patient was confined to bed.

The investigations were started one week after the operation had been performed. The patient's weight was 56 kg, her height was 160 cm. The calculated basal metabolic rate was 1,185 calories. The patient was fed by tube three times a day. Her daily caloric supply was 1,721 calories, exceeding by 45 % the calculated daily basal metabolic requirement. She received 83.7 g of protein daily, i.e. 1.5 g/kg body weight. The total caloric supply included 19.5 % protein, 34.0 % fat, and 46.5 % carbohydrates.

After a 2 week period of adaptation 100 mg of Primobolan Depot were given intramuscularly (figs. 9 and 10). A markedly positive nitrogen balance during the following 18 days resulted, the total nitrogen retention amounting to 30.6 g.

Discussion

We have in our study recorded nitrogen output through urine and stools but are well aware of the fact that losses also occur through the skin. Recently this aspect has been followed up in extraordinary well controlled metabolic experiments with direct determination of skin losses by B. Isaksson et al. (8). Their studies were correlated to determinations of exchangeable potassium as a measure of cell mass formation. According to these experiments the nitrogen losses through the skin normally amount to about 1 g a day.

Our patients were living under strictly controlled conditions and mostly confined to bed throughout the experimental periods. One might then assume that the nitrogen losses through the skin were constant and that a comparison between the effects of different anabolic steroids is possible under the given conditions.

In the clinic anabolic steroids are used to a great extent in patients with a certain degree of protein depletion, for instance in convalescence or postoperatively. It may then be justifiable to assume that the type of patient we have used for our studies is representative of the type of clinical case where anabolic steroids might be useful. In all our cases, it has been evident too that during the administration of different steroids there was a gain in weight, an improvement of their general condition and a disappearance of pressure sores etc.

The nitrogen retaining effect of anabolic steroids is dependent both on the caloric and the protein supply (9). If the dietary protein is to be utilized for the synthesis of body protein, there must be an adequate caloric supply at the same time. Nor may the non-protein dietary factors within the caloric supply be

lower than 800 to 900 calories (7). Studies on the protein metabolism employing N^{15} glycine and RISA (Radioiodine human serum albumin) have demonstrated that the protein metabolism is not influenced indirectly by anabolic steroids through inhibition of protein catabolism but that there is a direct influence which expresses itself in an acceleration of protein synthesis (12). These studies have also shown the extent of protein synthesis to be exclusively dependent on the degree of the caloric and protein supply.

All of our patients were fed by tube. Thus the intake of the previously computed amounts of protein and calories was absolutely reliable. By variation of the dietary protein and caloric contents we were also able to demonstrate the close relationship existing between the amount of the supply of protein and calories and the nitrogen retaining effect of anabolic steroids.

In cases 1 (first period) and 2 (to 1om) the daily protein and caloric supply had been kept at equal levels throughout the period of examination. 50 mg of Anadur each time produced the same mean daily nitrogen retention (table I, Nos. 1 and 11). When the protein and caloric supply was higher as in case 1 (second period) and in case 3, there was a considerable increase in the nitrogen retaining effect of Anadur which was almost equal in both cases (table I, Nos 7 and 16).

The importance of an increase in the protein supply for the nitrogen balance in one and the same patient is demonstrated by the results obtained in case 1 (first and second periods). While the non-protein nutritional factors were kept unchanged, the patient in the second period of the trial was given 51 % more protein than he had received in the first period. The nitrogen retention produced by Anadur in the second period was shown to exceed that of the first period by 58 %. Therefore an increase in the protein supply (51 %) resulted in an almost equal rise in the nitrogen retaining effect (58 %) (table I, Nos. 1 and 7).

When the caloric and the protein supplies were kept at constant levels (case 1, first period) 50 mg of Deca-Durabolin and 50 mg of Anadur produced approximately equal nitrogen retaining effects calculated per 24 hours (table I, Nos. 1 and 5). The difference between the respective total nitrogen retaining effects of both drugs during a period of positive nitrogen balance was due to the prolonged duration of the effect of Anadur. The effect of a 25 mg dose of Deca-Durabolin was found to be considerably weaker than both 50 mg of Anadur and 50 mg of Deca-Durabolin (table I, Nos. 1, 4 and 5).

An increase in the caloric and protein supply to the same patient (case 1, *first* and second periods) produced a rise in the nitrogen retaining action of 50 mg both of Deca-Durabolin and of Anadur by 60 and 58 % respectively. Thus both preparations showed *equal* increases in their activity (table I, Nos. 7 and 9). Androxan at a daily dosage of 15 mg likewise developed a considerably higher nitrogen retaining effect *when* the caloric and protein supply was raised (table I, Nos. 3 and 8).

One might of course assume that an increase of the caloric and protein supply

TABLE I

Case	Protein and caloric supply	No	Anabolic steroids used	Fig	Duration of the pos N balance (days)	Cumulative N balance (g)	Mean N retention (g/day)
1 1st period	Basal caloric requirements plus 29%, Protein 1.3 g/kg	1	Anadur 50 mg	1 B 2 B	28	49.0	1.7
		2	Androxan 10mg/day	1 C 3 C	7	6.6	0.9
		3	Androxan 15mg/day	1 D 3 D	8	5.9	0.7
		4	Deca-durabolin 25mg	1 E 2 E	18	18.3	1.0
		5	Deca-durabolin 50mg	1 F 2 F	20	30.9	1.5
		6	Dianabol 15mg/day	1 G 3 G	14	10.2	0.7
1 2nd period	Basal caloric requirements plus 55% Protein 1.65 g/kg	7	Anadur 50 mg	1 I 4 I	28	76.1	2.7
		8	Androxan 15mg/day	1 J 3 J	14	22.1	1.6
		9	Deca-durabolin 50mg	1 K 4 K	20	54.7	2.7
		10	Primobolan depot 100 mg	1 N 4 N	16	30.8	1.9
2	Basal caloric requirements plus 27% Protein 1.2 g/kg	11	Anadur 50 mg	5 A 6 A	28	48.1	1.7
		12	Androxan 10 mg/day	5 B 6 B	14	13.6	1.0
		13	Androxan 15 mg/day	5 C 6 C	14	10.3	0.7
		14	Dianabol 10 mg/day	5 D 6 D	14	11.4	0.8
		15	Dianabol 15 mg/day	5 F 6 F	11	9.6	0.8
3	Basal caloric requirements plus 33% Protein 1.7 g/kg	16	Anadur 50 mg	7 B 8 B	28	86.1	3.1
		17	Anadur 100 mg	7 C 8 C	42	97.3	2.3
		18	Anadur 50 mg	7 D 8 D	[1]21	47.7	2.3
4	Basal caloric requirements plus 45%, Protein 1.5 g/kg	19	Primobolan depot 100 mg	9 10	18	30.6	1.7

[1] Since the patient died the nitrogen balance was observed for a period of 21 days only

in itself might give a positive nitrogen balance That happened in fact, in case 1 where a positive balance can be seen after changing over to the higher caloric and protein level of period II This effect, however, ends after 2 weeks and only the administration of a steroid (Anadur) again gives a positive balance

It was demonstrated in this trial that under equivalent nutritional conditions 100 mg of Primobolan Depot exerted a lower mean nitrogen retaining effect than did 50 mg of Anadur or 50 mg of Deca-Durabolin (table I, Nos 7, 9, 10, 19)

Anadur, at a dosage of 100 mg, produced a positive nitrogen balance over a

period of 6 weeks The mean daily nitrogen retaining potency, however, was somewhat lower than that produced by 50 mg of Anadur in the same patient (table I Nos 16 and 17)

Since bronchopneumonia occurred in case 3 no more than a 21 day observation of the effect produced by the last administration of 50 mg of Anadur was possible (figs 7 and 8, table I, No 18) The results therefore cannot be compared with the others

In these investigations the nitrogen retaining effects of Androxan and of Dianabol have proved to be equal under the same nutritional conditions No difference was noted which might have been due to the dosages of Androxan or of Dianabol employed (table I Nos 3 6 14 and 15) This latter observation is consistent with the observations made in earlier studies on Dianabol (1)

The observed net gain in nitrogen balance is however within the limits of the generally accepted figures for nitrogen losses through the skin and this might imply that the orally administered anabolic steroids do not produce a real positive gain but only keep total losses and intake in balance

After administration of 100 mg of Primobolan Depot the nitrogen retaining effect lasted for 16 to 18 days (table I Nos 10 and 19) The nitrogen retaining effect produced by 25 mg of Deca Durabolin lasted 18 days (table I No 4) by 50 mg Deca Durabolin 20 days table I Nos 5 and 9) and the effect exerted by 50 mg of Anadur was demonstrable over a period of 28 days (table I Nos 1 7 11, and 16) It was shown that the duration of the nitrogen retaining effect is not dependent on the amount of calories or protein supplied, and was demonstrated to be equal both in the same patient under varying nutritional conditions as well as in different patients

The relationship between the amount of nitrogen retained computed as lean tissue, and the alterations in the weight of the patient may be seen in table II Since our patients were weighed once a week only, the weights recorded in this table can only be approximate values As may be seen from table II the gain in weight subsequent to the administration of a parenteral anabolic steroid is consistent on the whole, but with two exceptions with the computed amount of newly formed lean tissue Case 3 failed to develop the gain in weight theoretically computed, in spite of the large amount of lean tissue which should have formed (table II, No 16 figs 7 B and 8 B) The amount of calories supplied during this period presumably did not correspond to the actual requirements, for which reason the body fat may have been metabolized simultaneously with the formation of new muscular protein In case 1 the gain in weight following the administration of 50 mg of Deca Durabolin was twice the gain in lean tissue *which had been* computed theoretically (table II No 9 figs 1 K and 4 K) Presumably, this case is the opposite of the previous one Simultaneous fluid retention does not seem to have occurred

The gain in weight recorded after the administration of oral anabolic steroids actually exceeded the theoretically computed gain in lean tissue (table II Nos

TABLE II

Case	No	Anabolic steroid used	Fig	Total N retention (g)	Formation of new protein (g) (factor 6.25)	Formation of new lean tissue (g) (factor 5)	Alteration in weight (kg)
1	1	Anadur	1 B 2 B	49.0	306.3	1,531	+1.7
1st	2	Androxan	1 C 3 C	6.6	41.3	207	±0
period	3	Androxan	1 D 3 D	5.9	36.9	185	+0.7
	4	Deca durabolin	1 E 2 E	18.3	114.4	572	+0.3
	5	Deca durabolin	1 F 2 F	30.9	193.1	965	+0.5
	6	Dianabol	1 G 3 G	10.2	63.8	319	+2.1
1	7	Anadur	1 I 4 I	76.1	475.6	2,378	+1.9
2nd	8	Androxan	1 J 3 J	22.1	138.1	691	+0.6
period	9	Deca durabolin	1 K 4 K	54.7	341.9	1,709	+3.4
	10	Primobolan depot	1 N 4 N	30.8	192.5	963	+1.0
2	11	Anadur	5 A 6 A	48.1	300.6	1,503	+0.3
	12	Androxan	5 B 6 B	13.6	85.0	425	±0
	13	Androxan	5 C 6 C	10.3	64.4	322	+1.2
	14	Dianabol	5 D 6 D	11.4	71.2	356	±0
	15	Dianabol	5 F 6 F	9.6	60.0	300	+1.3
3	16	Anadur	7 B 8 B	86.1	538.1	2,690	±0
	17	Anadur	7 C 8 C	97.3	608.1	3,040	+2.6
	18	Anadur	7 D 8 D	47.7	298.1	1,490	+0.8
4	19	Primobolan depot	9 10	30.6	191.2	956	+1.5

3, 6, 13, and 15) Possibly, there was some degree of simultaneous water retention This assumption is strengthened by what has been said above the nitrogen gain does not in these cases exceed the probable nitrogen losses through the skin and so no material for the formation of new lean tissue, corresponding to the gain in weight, is available The duration of our clinical trials on these steroids, however, has been so short, and the cases tried so few that no definite statements on the results achieved are possible

The results given in table II confirm the observation made earlier (13) that caution is necessary in the assessment of the action of anabolic steroids when it is based exclusively on alterations in weight, which may also be caused by changes in water balance and fat deposition

The diet fed by tube, which was prepared from milk, glucose, Intralipid, and Meritene, and which, in one of our cases, was given over a period of 347 days, produced no gastrointestinal disturbances whatsoever in any of our subjects

Summary

The investigations performed under the above described strictly controlled conditions have demonstrated that without an adequate supply of protein and calories, anabolic steroids will produce no nitrogen retaining effect and the extent of the supply of calories and protein is of fundamental importance for the degree of nitrogen retention

The mean daily nitrogen retaining activities of 50 mg of Anadur and Deca Durabolin, respectively, were found to be equal Anadur, however, produced a longer lasting effect The total amount of nitrogen retained during a period of positive nitrogen balance was therefore higher

Deca Durabolin at a dosage of 25 mg produced a considerably lower effect, and 100 mg of Primobolan Depot showed a somewhat lower mean nitrogen retaining activity per day than did 50 mg of Deca Durabolin and Anadur respectively

The nitrogen retaining effect exerted by Androxan was virtually equal to that produced by Dianabol These preparations however are less effective than the parenterally administered steroids and the daily retained amount of nitrogen does not exceed about 1 g a day which corresponds to the generally accepted figure for possible losses through the skin

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TABLE II

Case	No.	Anabolic steroid used	Fig.	Total N retention (g)	Formation of new protein (g) (factor 6.25)	Formation of new lean tissue (g) (factor 5)	Alteration in weight (kg)
1	1	Anadur	1 B 2 B	49.0	306.3	1,531	+1.7
1st	2	Androxan	1 C 3 C	6.6	41.3	207	±0
period	3	Androxan	1 D 3 D	5.9	36.9	185	+0.7
	4	Deca-durabolin	1 F 2 F	18.3	114.4	572	+0.3
	5	Deca-durabolin	1 F 2 F	30.9	193.1	965	+0.5
	6	Dianabol	1 G 3 G	10.2	63.8	319	+2.1
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3, 6, 13, and 15). Possibly, there was some degree of simultaneous water retention. This assumption is strengthened by what has been said above: the nitrogen gain does not in these cases exceed the probable nitrogen losses through the skin, and so no material for the formation of new lean tissue, corresponding to the gain in weight, is available. The duration of our clinical trials on these steroids, however, has been so short, and the cases tried so few that no definite statements on the results achieved are possible.

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From King Gustaf V Research Institute Stockholm Sweden

The Influence of Different Methods of Dissociating the Rheumatoid Factor, Studied by Ultracentrifugation, Sheep-cell Tests and Immunoelectrophoresis

By

NANNA SVARTZ, HELMUT SCHATZ and STIG HEDMAN

The basis for the investigations to be reported here was our experience of the splitting of the rheumatoid factor with the aid of ammonium sulphate In 1962 it was demonstrated that fractionation of the rheumatoid factor (RF) by ammonium sulphate added to 1 23 M provoked a breakdown of the factor without loss of its hemagglutinating capacity Ultracentrifugation before and after precipitation with $(NH_4)_2SO_4$ showed that the 18 to 19 S peak present in the original serum, generally disappeared altogether or at least diminished considerably New peaks were formed which showed sedimentation coefficients of about 14—16 S Even if the 18—19 S peak had totally disappeared the redissolved precipitate gave a positive sheep cell test with about the same titer as the original serum (4)

Earlier experience had shown that disintegration of the rheumatoid factor usually renders negative an otherwise positive hemagglutination test This was the case in trials with urea and mercaptoethanol for instance In the present study we have compared the influence on the rheumatoid arthritis serum and particularly on the rheumatoid factor of precipitation in cold, fractionation with ammonium sulphate and treatment with cysteine or mercaptoethanol The changes were studied by aid of ultracentrifugation, sheep cell tests and immunoelectrophoresis

Material and methods

Analytical ultracentrifugation was performed on untreated *serum* from rheumatoid arthritis patients as well as on fractions of serum obtained by fractionation in cold *or with ammonium sulphate* or after treatment with cysteine or mercaptoethanol

The hemagglutination test (sheep-cell test or Waaler Rose's test) was made on every fraction

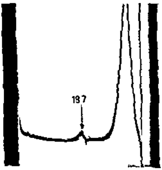
The different serum fractions were also studied by immunoelectrophoresis

As to the methods used the following points warrant mention

Submitted for publication July 27 1964

Figs 1—6 The results obtained by the methods mentioned are illustrated by the following data (Only one series can be demonstrated)

Ultracentrifugation	Hemagglutin	Immunoelectrophoresis



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Rheumatoid arthritis (RA) serum (untreated) ultracentrif



I RA serum II Anti human serum III Normal serum

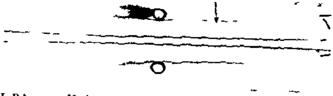
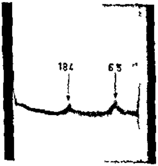


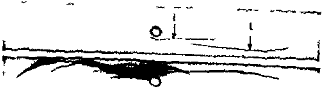
Fig 1

I RA serum II Anti- IM III Normal serum



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Cold precipitate from RA serum ultracentrif



I Cold precipitate II Anti human serum III Normal serum

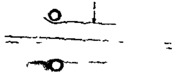


Fig 2

I Cold precipitate II Anti IM III Cold precipitate (conc twice)

For ultracentrifugation an ultracentrifuge type Beckman Spinco E was used The ultracentrifuge was usually run for two hours at 59,000 to 60,000 r p m Every sample was ultracentrifuged in six different dilutions for determination of the sedimentation constant (5)

Fractionation in cold (+ 4°) was performed according to the method earlier described (6)

Fractionation with ammonium sulphate added to 1 23 M was performed according to the method described in Acta med scand 1962 (4) and elsewhere (7) The precipitates were redissolved in saline The supernatants were usually not examined

For investigation of the effect of cysteine, the cysteine reagent (an aqueous solution of cysteine hydrochloride, 0,1 M, and sodium hydroxide 0 2 M) was added to the serum in the proportion 1 5 For mercaptoethanol the method of Deutsch and Morton (1) was used

The hemagglutination test of Waaler-Rose was performed by the method as modified by Svartz and Schlossmann (8)

For immunoelectrophoresis the modification of Scheidegger and Heremans et al was used (2, 3) The determinations were made with horse immune serum against human serum ("anti human serum") and with anti $beta_{2M}$ serum here called anti $gamma_{1M}$ serum Rabbit immune sera against the following human serum components were also tried gamma (7 S) and the system of gamma globulin components ("immune globulins") These antisera were obtained from the Central Laboratory of Blood Transfusions Red Cross Amsterdam Besides a special antiserum against the purified rheumatoid factor was produced here by immunizing rabbits

Results

As may be seen from fig 1 the *original rheumatoid serum* showed two peaks 18,7 S and 7 S on ultracentrifugation

The hemagglutination test was strongly positive (titer 1 4 096)

Immunoelectrophoresis with anti human serum showed no definite divergence from normal serum There was no significant increase of the $gamma_{1M}$ precipitation line, even when anti $gamma_{1M}$ or anti RF was used

Ultracentrifugation of the *cold precipitate* from serum, redissolved in saline to the same volume as the original serum, showed two peaks, viz 18 4 S and 6 5 S The 6 5 S peak is about a tenth of the size of that for the non precipitated serum (fig 2)

The hemagglutination test was strongly positive (titer 1 4, 096)

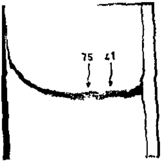
Immunoelectrophoresis on the redissolved cold precipitate performed with anti human serum revealed the presence of γ_1 macroglobulin and γ_2 (7 S) globulin (In another electrophoresis traces of α_2 lipoprotein and albumin were seen) The γ_1 precipitation, which was rather weak with anti human serum, was stronger when anti γ_{1M} serum was used

Fractionation with *ammonium sulphate* (1,23 M) showed the following results With ultracentrifugation an insignificant peak of 18—19 S was sometimes found in the precipitate, but usually it totally disappeared as found by Svartz and Hedman (4) The ultracentrifugogram showed in the case, fig 3 no protein with a sedimentation coefficient of 18—19 S in the redissolved precipitate but two new peaks of 16 S and 12 S Peak of 7 S was also found

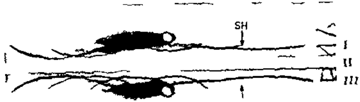
In spite of the fact that the macroglobulin had disappeared the hemagglutination test turned out positive with the same titer as in the original serum

Ultracentrifugation Hemag glut n

Immunoelectrophoresis

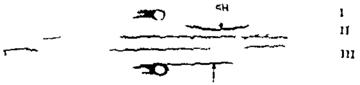


Cys e ne f act on of R A se um ul racen r f



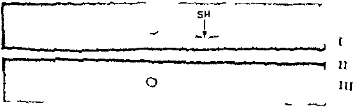
I Cys e ne fract on of RA serum II Ant human III O ginal RA serum

neg



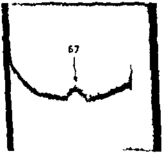
I Cys e ne f ac on of RF II An III Or g nal RA serum

neg



I Cyste ne f act on of RF II Ant RF III 7 S (γ)

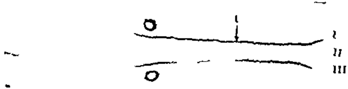
Fig 4



7 S g b l n sola ed here ul racen nf



I 7 S γ gl b l n mmercial II An h man III N rmal serum

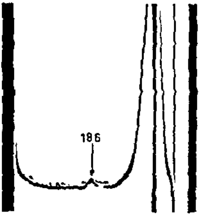


I S-γ globul n (isolat d here) II Ant III N rmal serum

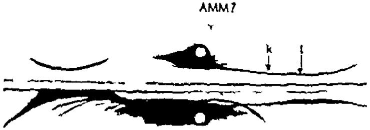
Fig 5

Ultracentrifugation	Hemag glutin	Immunoelectrophoresis

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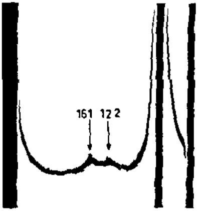


R A serum (untreated)

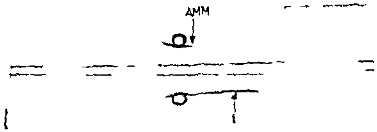


I $(NH_4)_2SO_4$ fraction of R A serum II Anti human III Ori serum

1 409(



ammonium sulphat precipitate
om RA serum ultracentrif



I $(NH_4)_2SO_4$ fraction of R A serum II Anti IM III Original R serum

ig 3

Immunoelectrophoresis with anti human serum (fig 3) showed that many more proteins were precipitated with $(NH_4)_2SO_4$ than with cold fractionation Of the different γ globulins only the γ_{1A} and γ_2 globulins could be identified with certainty, while the γ_1 macroglobulin precipitation line could not be distinguished A distinct albumin line was also found Close to the well there is a short precipitation line that seems to be localized to the anodic part of the γ_{1M} line It might perhaps derive from one of the new globulins Using an anti γ_{1M} serum it was established that the cathodic part of the normal γ_{1M} precipitation line had disappeared while the anodic part of it was still there These results were confirmed with other sera It is possible that the short precipitation lines correspond to the 16 S or 12 S peaks observable in the ultracentrifugogram

The results of treatment of rheumatoid serum with *cysteine* or *mercaptoethanol* are of considerable interest (fig 4) As found by others ultracentrifugation showed no macroglobulin at all Only peaks of 7 5 and 4 1 S were seen

The hemagglutination test was negative

On immunoelectrophoresis with anti human serum a strong but short precipitation line was found in the γ_{1M} area Using anti γ_{1M} serum a strong line was seen in the cathodic part of the γ_{1M} area Now from the ultracentrifugation we knew that we were dealing here with a 7—8 S globulin Therefore it seemed desirable to compare the result with that obtained with 7 S globulin of the common type The 7 S globulin which appeared after cysteine treatment of RF did not show any reaction with a specific anti γ_2 (7 S) serum known from other experiments to precipitate ordinary 7 S globulin selectively Moreover, anti γ_{1M} or anti RI serum gave precipitation with cysteine 7 S but not with ordinary 7 S (fig 4 third picture) Immunoelectrophoresis was performed as a control with ordinary 7 S globulin (so called human gamma globulin') and anti 7 Sγ globulin (fig 5) Strong precipitation appeared It should be added that, on use of commercial 7 S globulin and anti γ_{1M} serum, a slight trace of precipitation was found in the γ_{1M} area Anti-human serum likewise revealed the ordinary 7 S gamma globulin to contain traces of γ_{1M} components besides the main fraction (fig 5) Thus, there was not solely 7 S material in the human gamma globulin

The rheumatoid factor, purified by cold fractionation and chromatography, when tested by the above methods, gave the following results

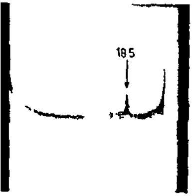
Ultracentrifugation showed, as reported in several papers (5 7) an isolated peak between 18 and 19 S, in this case 18 5 S Thus, the RF appeared to be in a pure state (fig 6)

The hemagglutination test was strongly positive

As judged by the result of immunoelectrophoresis of RF, using anti human serum or anti $gamma_{1M}$ serum our RF seemed to be also immunologically in a pure state and to belong to the $gamma_{1M}$ globulin fraction

At this stage of the investigations it was obviously of interest to study by immunoelectrophoresis the reaction be-

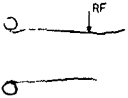
lltracentrifugation	Hemag glutin	Immunoelectrophoresis
	1 8192	



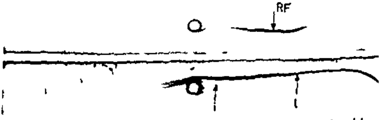
I purified (isolated here) ultra ntrif



I RI (purified) II Anti human III Normal serum



I RI (purified) II Anti γ1M III Normal serum



I RF (purified) II Anti human III 7 S γ globulin (isolated here

g 6

mbols

- ı ₁ macroglobulin (γ1M)
- k γ1A
- l γ₂ (7 S globulin)
- MM – $(NH_4)_2SO_4$ fraction of γ1M
- SH Cysteine fraction of γ1M
- RI RI macroglobulin (belonging to 1M group)

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Ultracentrifugation showed, as reported in several papers (5, 7) an isolated peak between 18 and 19 S in this case 18 5 S Thus, the RF appeared to be in a pure state (fig 6)

The *hemagglutination* test was strongly positive

As judged by the result of immunoelectrophoresis of RF, using anti human serum or anti gamma$_{1M}$ serum, our RF seemed to be also immunologically in a pure state and to belong to the gamma$_{1M}$ globulin fraction

At this stage of the investigations it was obviously of interest to study by immunoelectrophoresis the reaction be

tween the purified RF *macroglobulin* and anti-RF serum. In order to get anti-RF serum, rabbits were immunized by injecting RF intramuscularly *together with* Freund's adjuvant (without bacteria). Three and four weeks later intravenous injections of RF were given. The immunization gave rise to a strongly precipitating antiserum.

Grabar has pointed out that the best method hitherto of detecting impurities" in, for instance, protein fractions is to produce antibodies against the material. We now tested the purified RF against the anti-RF serum obtained by immunization. The RF in question had shown only one peak on ultracentrifugation and one precipitation line on immunoelectrophoresis. The immunoelectrophoresis performed with RF and anti-RF showed that the RF solution contained traces of 7 S gamma globulin and also traces of another globulin, the nature of which is not yet fully clarified. It has also been possible to compare RF-macroglobulin with other macroglobulins by the purification method. These studies are in progress as are also the studies of the disintegration products of RF.

Conclusions

Fractionation of rheumatoid serum in cold effects a surprisingly good purification of the rheumatoid factor. Another important advantage is that precipitation in cold does not change the sedimentation coefficient of the RF macroglobulin. The hemmagglutination test remains positive.

The results of fractionation with ammonium sulphate show that this method should not be used for purification of *macroglobulins*, in view of the pronounced reduction of the macroglobulin to which it gives rise. Remarkably *enough, the precipitate from rheumatoid* arthritis serum shows a positive hemagglutination test with about the same *titer as the original serum, in spite of* the splitting of RF.

Immunoelectrophoresis of the gamma$_M$-*macroglobulin as split by ammonium* sulphate showed that the electrophoretic mobility had changed. Thus, the ordinary gamma$_M$ precipitation line did not appear, but instead a much shorter and more anodically situated precipitation line was demonstrable.

Cysteine and mercaptoethanol provoke a strong disintegration of the RF-macroglobulin. Thus, a breakdown to about 7 S occurred and no higher sedimentation coefficient could be observed. The hemagglutination test turned out negative. Immunoelectrophoresis shows a pronounced difference between the 7—8 S globulin, formed by treating serum with cysteine or mercaptoethanol and "normal" 7 S gamma globulin. As shown by Deutsch and others the 7 S component formed by treating serum containing γ_1 macroglobulin with cysteine has about the same percentage of carbohydrate as the *macroglobulin*, while the ordinary 7 S contains only 2—4 % carbohydrate. The result with *cysteine shows* that the hemagglutination provoked by RF is not caused by the carbohydrate content of this factor as has been claimed, since the percentage is the same before and after splitting (about 10 %) but nevertheless the Waaler Rose test turns out negative after the splitting with cysteine.

It is evident that the differences between the different 7 S globulins are of great interest for the discussion of the nature and formation of the rheumatoid factor and other macroglobulins

The RF macroglobulin purified according to our methods, shows only one isolated peak in the ultracentrifugogram and only one precipitation line in immunelectrophoresis, which is more clearly demonstrable, if the RF is concentrated before testing

In spite of the above mentioned tests of purity, the presence of traces of other substances in the purified RF could be detected by immunizing rabbits with this same RF These impurities can be removed by absorption

Summary

Comparisons were made between the results obtained by ultracentrifugation, hemagglutination (sheep cell test) and immunoelectrophoresis on serum from rheumatoid arthritis patients before and after fractionation of the serum

Cold fractionation did not change either the sedimentation coefficient on ultracentrifugation or the hemagglutination test or the immunoelectrophoretic pattern of the rheumatoid factor macroglobulin

Fractionation with ammonium sulphate dissociates the macroglobulin and at the same time changes its sedimentation coefficient The hemagglutionation remains unchanged Instead of the normally occurring $gamma_{1M}$ (beta$_{2M}$) precipitation line only a short, more anodically situated line appears

After treatment of rheumatoid serum with cysteine or mercaptoethanol the RF macroglobulin totally disappeared and no macroglobulin was demonstrable by ultracentrifugation Instead, a strong 7—8 S peak appeared The Waaler Rose test turned out negative On immunoelectrophoresis a precipitation line was found corresponding to the position of that part of $gamma_{1M}$ which is located most cathodically This precipitation line had a quite different position than the line of ordinary 7 S gamma globulin Other differences could be established too

The RF isolated by cold precipitation and chromatography gave rise to a single 18—19 S peak on ultracentrifugation and to an isolated precipitation line in $gamma_{1M}$ on immunoelectrophoresis The sheep cell test was positive

By immunization of rabbits with the RF macroglobulin it could be demonstrated that in spite of the results of the above mentioned tests, traces of other substances *often exist in* the purified RF fraction They have no influence on the tests reported here, but can be removed by absorption

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Free Fatty Acids of Plasma During Hypoxia and Hypercapnia in Dog

By

Lars Orö

The adrenergic neurohormones norepinephrine and epinephrine, stimulate the mobilization of free fatty acids (FFA) from adipose tissue in vitro (20, 36) and increase the concentration of FFA in plasma (9 14, 19 21 22 24) There is also a rise of the FFA level in plasma during many conditions with increased activity in the sympathetic nervous system such as mental stress (5 7) long term exercise (3) cold stress (29) and hypoglycemia (2 16 35)

The effects of carotid occlusion and central vagal stimulation on the FFA level in plasma and the blood pressure were recently studied in the dog (15) The results suggested that the baroceptor reflexes were of no importance for the mobilization of FFA to plasma The chemoreceptor reflexes are known also to influence the activity in the sympathetic nervous system and are of importance for homeostasis in the body (17 23 33) In this investigation the possible role of the chemoreceptor reflexes in the mobilization of FFA was studied by means of producing respiratory hypoxia and hypercapnia in dogs Sodium cyanide, which is known to cause histotoxic hypoxia and to stimulate the chemoreceptors (8 10, 12 28), was also infused intravenously

Methods

Experimental Mongrel dogs weighing between 19—26 kg were used After fasting overnight they were anesthetized with Nembutal® (Abbott) and the trachea was intubated The dogs were breathing spontaneously during the experiments The blood pressure was measured continuously with an Elema Schönander pressure transducer (EMT 490 A) from a teflon catheter inserted into one femoral or brachial artery The pressure transducer was filled with saline and no heparin was injected into the animals The dogs were given different gas mixtures from an AGA gas mixer (MDNA 20 3000) by means of a small catheter introduced into the tracheal tube

Respiratory hypoxia was produced in 8 dogs In the beginning of the experiments a gas mixture of 50 % O_2 in N_2 was given (1 5 l O_2 and 1 5 l N_2 per minute) The oxygen con

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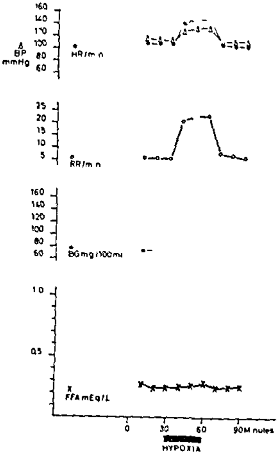


Fig. 1. Free fatty acids of plasma (FFA), blood glucose (BG), respiratory rate (RR), mean blood pressure (BP) and heart rate (HR) in anesthetized dogs during respiratory hypoxia. The mean values from the experiments in 8 dogs are given.

centration was then lowered during a thirty minute period so that the respiratory frequency increased three or four times. The composition of the gas mixture was continuously adjusted to keep the respiratory frequency as constant as possible (0—0.5 l O_2 and 2.5 l N_2 per minute). After this period the initial gas mixture of 50 % O_2 in N_2 was given until the end of the experiment.

Respiratory hypercapnia was produced in 6 dogs. The animals received O_2 (1 l/min.) in the tracheal tube during the whole experiment. CO_2 was added (1—3 l/min.) during a thirty minute period so that the respiratory frequency increased three or four times.

Sodium cyanide dissolved in saline was infused i.v. into 6 dogs at a constant rate of 10 μg/kg body weight/minute for a period of thirty minutes. The animals only received air in their tracheal tubes.

Analytical. Arterial blood samples were withdrawn into heparinized syringes. The FFA were determined by the method of Dole (9) as modified by Trout et al. (34). Blood glucose was determined with glucose oxidase according to Marks (25). The P_{O_2} and P_{CO_2} in arterial blood were determined according to Severinghaus and Bradley (31). The statistical analyses were performed as described by Snedecor (32).

Results

Respiratory hypoxia

The respiratory rate markedly increased in all dogs during the administration of the gas mixture with low oxygen tension. The mean increase was from 6 to 23 breaths per minute (fig. 1 and table I). The mean blood pressure and the mean heart rate increased from 115 to 135 mm Hg and from 110 to 143 beats per minute respectively. The tension of oxygen and of carbon dioxide in arterial blood were determined in three experiments. The mean P_{O_2} decreased from 131 to 25 mm Hg and the mean P_{CO_2} from 75 to 39 mm Hg (table II). The concentration of free fatty acids in plasma remained almost unchanged at a mean level of 0.25 mEq/l (fig. 1 and table I). The blood glucose concentration was unchanged at a mean level of 74 mg/100 ml.

Respiratory hypercapnia

The respiratory rate increased in all dogs during the administration of carbon dioxide. The mean increase was from 7 to 23 breaths per minute (fig. 2 and table I). No significant change of the mean blood pressure or the mean heart

TABLE I Changes of the concentration of plasma FFA and blood glucose respiratory rate mean blood pressure and heart rate during respiratory hypoxia and hypercapnia and during infusion of sodium cyanide in dog The figures give the mean values and the standard errors of the mean and are calculated on the individual changes from the values immediately before the different procedures

		FFA concentration (mEq/l)			Blood glucose concentration (mg/100 ml)			Respiratory rate (breaths/min)	Mean B P (mm Hg)	Heart rate (beats/min)
	After (min)	10	20	30	10	20	30	20	20	20
Respiratory hypoxia 8 dogs	Mean change	0.00	0.02	0.05	—1	—1	0	*17	*20	*33
	S E of mean	±0.01	±0.02	±0.04	±1	±1	±1	±4	±3	±6
Respiratory hypercapnia 6 dogs	Mean change	0.01	0.00	0.01	18	22	*24	*16	—5	15
	S E of mean	±0.03	±0.03	±0.01	±7	±10	±9	±2	±6	±8
Infusion of NaCN 40 µg/kg min) 6 dogs	Mean change	0.06	0.09	0.06	—2	17	*52	*10	*20	*42
	S E of mean	±0.02	±0.04	±0.04	±3	±6	±18	±1	±5	±7

* and * indicate that the statistical significance (P) is < 0.05 0.01 and 0.001 respectively

TABLE II The tension of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) in arterial blood before and during respiratory hypoxia and hypercapnia in dog The blood samples were taken about 10 min before and 20 min after the administration of the different gas mixtures started The figures give the mean values and the standard errors of the mean

		P_{O_2} (mm Hg)		P_{CO_2} (mm Hg)	
		Before	During	Before	During
Respiratory hypoxia 3 dogs	Mean value	131	25	75	39
	S E of mean	±39	±7	±10	± 8
Respiratory hypercapnia 3 dogs	Mean value	126	134	63	235
	S E of mean	± 7	±6	±12	±45

rate was observed (fig 2 and table I) The tension of oxygen and of carbon dioxide in arterial blood were determined in three experiments The mean P_{CO_2} increased from 63 to 235 mm Hg and the mean P_{O_2} from 126 to 134 mm Hg (table II) The concentration of FFA in plasma remained unchanged at a mean

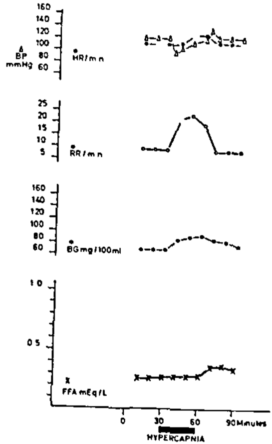


Fig 2 Free fatty acids of plasma (FFA) blood glucose (BG) respiratory rate (RR) mean blood pressure (BP) and heart rate (HR) in anesthetized dogs during respiratory hypercapnia The mean values from the experiments in 6 dogs are given

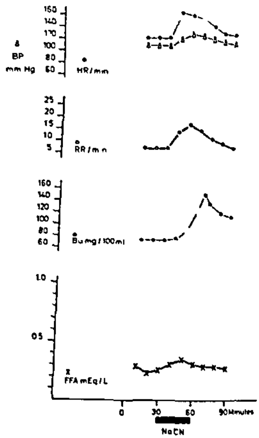


Fig 3 Free fatty acids of plasma (FFA), blood glucose (BG) respiratory rate (RR), mean blood pressure (BP) and heart rate (HR) in anesthetized dogs during infusion of sodium cyanide (NaCN), 40ug/kg body weight/minute The mean values from the experiments in 6 dogs are given

level of 0 27 mEq/l (fig 2 and table I) The mean blood glucose concentration increased from 68 to 92 mg/100 ml ($p < 0.05$)

Infusion of sodium cyanide

During the first 20 minutes of the infusion of sodium cyanide the mean respiratory rate successively increased from 7 to 17 breaths per minute (fig 3 and table I) The mean respiratory rate then tended to decrease and in two of the six dogs respiration stopped about 5 minutes before the end of the infusion These two dogs were given artificial respiration with air until they started to breath spontaneously 10—15 minutes later The mean blood pressure and the mean heart rate increased from 110 to 130 mm Hg ($p < 0.01$) and from 122 to 164 beats per minute ($p < 0.001$) respectively (fig 3 and table I) No significant change of the concentration of FFA in plasma, form the initial mean level of 0 26 mEq/l, was observed (fig 3 and table I) The mean blood glucose level increased from 72 to 124 mg/100 ml ($p < 0.05$) during the infusion of sodium cyanide

Discussion

McElroy and Spitzer previously reported that respiratory acidosis and hypoxia were stimuli which increased the concentration of FFA in plasma in dogs (26, 27), but in this investigation no changes in the arterial level of FFA were observed during similar conditions. The FFA level was also unchanged when histotoxic hypoxia was produced by means of infusion of sodium cyanide.

There are reasons to believe that the discrepancy in the results is explained by important differences in the techniques. McElroy and Spitzer determined the FFA with the original method of Dole (9). With this method the FFA titration values are influenced by lactic and pyruvic acid but not with the modified method described by Trout et al (33). As the production of lactic and pyruvic acid is known to increase during hypoxia and hypercapnia (11–13) the FFA were therefore analyzed according to Trout in this investigation. Furthermore McElroy and Spitzer used a respiratory apparatus to administer the different gas mixtures and the respiratory changes could therefore not be studied as in this investigation.

Anesthetic agents like pentobarbital are known to depress cardiovascular reflexes (30) and the transmission of nerve impulses in autonomic ganglia (18) and may also inhibit the mobilization of free fatty acids to plasma. However the FFA level in pentobarbital anesthetized dogs rapidly increases when catecholamines are infused intravenously (15, 19) or when one of the sympathetic chains is electrically stimulated (Orö, unpubl. observation).

In this investigation marked changes in the arterial P_O and P_{CO} were produced. The respiratory changes indicated that the chemoreceptor reflexes were activated. The sympathetic nervous system was also stimulated during the respiratory hypoxia as well as during the infusion of sodium cyanide for the blood pressure and heart rate increased (4, 23). However no simultaneous change in the arterial level of FFA was observed. The results seem to show that the chemoreceptor reflexes could be activated in these preparations without any change of the FFA level in plasma.

The circulatory changes during conditions such as heavy exercise are known to influence the concentration and flux of FFA in plasma (6). The increased heart rate and blood pressure during the respiratory hypoxia and the infusion of sodium cyanide may therefore have been of importance for the unchanged level of FFA. However, no significant changes of heart rate or blood pressure occurred during the respiratory hypercapnia and generally the rate of FFA turnover has been found to be proportional to its concentration (22). It therefore appears possible that the chemoreceptors play no role in the mobilization of FFA to plasma.

In accordance with other investigations including that of McElroy and Spitzer (13, 26) the author found that the blood glucose level tended to increase during the respiratory hypercapnia. McElroy and Spitzer also observed an increased blood glucose level during respiratory hypoxia (27). When the chemoreceptors in the cat were locally stimulated with cotconsum a

nicotinemimetic substance, the levels of glucose and insulin in the blood increased (1) However in this investigation the blood glucose level only increased when hypoxia was produced by infusion of sodium cyanide During respiratory hypoxia the blood glucose concentration was unchanged although the mean P_{O_2} decreased to a level as low as 25 mm Hg These data taken together suggest that maybe only very strong stimuli on the chemoreceptors cause an increase of the blood glucose level

Summary

The effect of hypoxia and hypercapnia on the respiration and the concentration of free fatty acids in plasma (FFA) has been studied in anesthetized dogs When gas mixtures of low oxygen tension or high carbon dioxide tension were administered by tracheal tube the respiratory rate increased three or four times The respiration was also stimulated by infusion of sodium cyanide No significant changes in the arterial levels of FFA were observed during these procedures The blood glucose level increased during respiratory hypercapnia and during the infusion of sodium cyanide but was unchanged during respiratory hypoxia

The possibility that the chemoreceptor reflexes are not of importance for the mobilization of FFA to plasma is discussed

Acknowledgement

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The Acute Radiation Syndrome — the Importance of the Gastro-intestinal Injury in the Catabolism and Distribution of Serum-albumin

By

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It has been quite clearly shown that *some organ* systems are particularly sensitive to ionising radiation namely *the gastro intestinal tract*, the immune body forming and the haematopoietic systems and the sexual organs (7, 8)

A decrease of the serum albumin concentration with inversion of the albumin/globulin ratio after whole body irradiation has been demonstrated by several authors (9, 19, 13, 11 30)

As albumin catabolism normally occurs mainly in the gastro-intestinal tract (2 27 28), the intestinal tract damage induced by ionising radiation could cause a pathologically increased loss of albumin with resulting increased catabolism and hypoalbuminaemia — hypoproteinaemia Such an effect has been demonstrated (4)

It has now been sought to obtain more extensive knowledge of the albumin catabolism after whole body irradiation using varying doses but especially LD_{50} and of the distribution of albumin in the body after high doses of radiation Another object has been to study the variations in the plasma volume after irradiation, and to find out whether they play any compensatory role in the hypoalbuminaemia Further, it seemed possible to us that a study of the initial disappearance of ^{131}I albumin would help to reveal any disturbances in the general capillary permeability

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Material and methods

Rabbits weighing 2 5—4 5 kg were used in the experiments They were apparently healthy The rabbits used for metabolic and compartmental studies received a standard diet and were kept in special metabolic cages in which quantitative collection of urine and faeces could be made

Nineteen rabbits were subjected to metabolic studies 14 had received varying doses of ionising radiation and 5 were used as controls In 8 animals the distribution of ^{131}I albumin in different organs was studied

3 served as controls, and 5 were examined after varying radiation doses Repeated plasma volume determinations with ^{131}I-albumin were made after irradiation in 15 rabbits Initial disappearance was determined in 15 animals (16 experiments), 6 of which were used as controls

The animals were given whole body irradiation in a gammatron (^{60}Co, Super G Atomic Energy, Canada Ltd) at the Radium hemmet The radiation dose (275—2,500r) was checked with a BG dosimeter (Sievert)

Homologous albumin prepared from rabbit serum by zone electrophoresis in polyvinyl chloride was used in the experiments Labelling with ^{131}I was made by McFarlane's (17) technique The content of free ^{131}I (i e non protein bound) in the preparations did not exceed 1 % The amount of radioactivity was ~ 50 μc per mg of albumin, immediately following the labelling

All the animals were given sodium iodide in their food daily for two days before and throughout the period of investigation, to prevent thyroid uptake of released ^{131}I

70—100 μC of ^{131}I-albumin were given intravenously about 18 hours after irradiation to the rabbits used for metabolic and distribution studies In those rabbits that received 1 600—2,500r, the injection was given immediately after the irradiation For the repeated plasma volume determinations, gradually increasing doses of ^{131}I albumin were used so as to obtain accurate values

Nine minutes after the injection of ^{131}I albumin into a marginal ear vein, blood samples were in each animal drawn from the vein of the other ear The plasma volume was calculated from the radioactivity in this sample by the conventional isotope dilution technique All the blood samples consisted of 0 5 ml They were centrifuged immediately without addition of heparin, and the plasma was then pipetted off and weighed with a precision of 0 1 mg

In the 19 rabbits in which the albumin catabolism was examined the plasma activity and the whole body activity were determined daily during 10—14 days or up to the death of the animals Urine and faeces were collected daily and assayed for radioactivity, the faeces after homogenization Radioactivity was measured in a Tracerlab well crystal scintillation counter and Super scaler SC-18A or a Versamatic II Scaler Spectrometer All the measurements were corrected for background activity and radioactive decay by reference to a standard

The whole body activity was measured with a scintillation counter with a wide-angle clinical collimator placed at a fixed distance To ensure satisfactory geometry, the animals were kept in a box which allowed only small movements of their bodies A plastic bottle of 'rabbit' size filled with water and 100 μC of ^{131}I albumin was used as a standard in these experiments

Total protein in plasma was determined by Kjeldahl s technique and the albumin concentration by paper electrophoresis (21), initially in all the animals and continuously in 14 of those which were used for metabolic analyses and in those studied by repeated plasma volume determinations The haematocrit was determined initially and therafter in connection with sampling for paper electrophoresis

The distribution of ^{131}I albumin in different organs was studied in 3 controls (table V) In 5 animals which had been irradiated with varying doses, the radioactivity in the different organs was measured after they had been killed or had died spontaneously

Among the 15 animals in which repeated plasma volume determinations with ^{131}I albumin were made, 800r (LD_{50}) had been given to 8 and 1,200r to 7 The determinations were performed on peripheral venous blood In 2 controls 3 of the 800r group, and 3 of the 1 200r group, simultaneous sampling was done from central veins and arteries The venous haematocrit was determined in connection with the measurements Total blood volume and red cell volume were not calculated on the basis of the peripheral haematocrit values as these are subject to considerable uncertainty The albumin content in plasma was determined in all the animals in connection with the measurements of volume

TABLE I Control animals Data on plasma volumes, intravascular albumin and degradation of albumin according to the two methods used In rabbit no 5 two investigations were made

Rabbit no	Weight (kg)	P V	P V /kg	Haematocrit	Intravascular albumin (total g)	(g/kg)	Degradation in % of i v pool: Campbell	Matthews	Degradation g	g/kg
1	4 38	150	34 3	36	7 0	1 60	14 7	15 5	1 03—1 08	0 23—0 25
2	2 65	104	39 3	34	4 9	1 85	20 8	19 4	0 95—1 02	0 36—0 39
3	2 42	87	35 9	32	3 4	1 40	21 0	16 9	0 58—0 71	0 24—0 29
4	3 59	158	44 0	32	6 5	1 81	24 5	25 2	1 40—1 60	0 39—0 44
5 a	3 38	104	30 8	34	4 1	1 21	29 7	25 5	1 05—1 22	0 31—0 36
5 b	3 18	104	32 7	33	4 5	1 41	27 0	24 0	1 08—1 21	0 34—0 38

The initial disappearance from the blood of ^{131}I albumin given at various times after irradiation with 800 1200r was determined in 9 animals Six others served as controls In some rabbits samples were drawn peripherally from the ears and centrally through a catheter in the femoral vein and/or artery The samples were taken within 9 and 12 minutes of the injection of ^{131}I albumin thereafter every 10 minutes *for 2 hours and* every 15 or 20 minutes during the next 6 hours

The albumin degradation was determined by the method of Campbell et al (5) where the catabolic rate is expressed as

$$\frac{\text{excreted activity in urine and faeces}}{\text{mean activity in plasma}} \text{ per 24}$$

hour and by Matthews (16) *method in* which graphical analysis of the plasma activity curves gives the degradation under steady state conditions The obtained percentage values thus refer to the intravascular albumin pool The quantitative values in g per day and g per kg per day given in table II were calculated from the original values and any decrease of the intravascular pool that may have occurred during the experiment was not taken into consideration In the 800r group and in one animal in the 400r-group quantitative estimations were also made from the specific activity of plasma Using the equilibrium time method the extravascular distribution could be calculated on the basis of graphical analysis in these metabolic studies

In assessing the capillary transfer rates it seems appropriate to use only the expression $\Sigma\lambda_n C_n$ derived from the graphical analysis which is the sum of $\lambda_1 C_1 + \lambda_2 C_2$ where λ_1 and λ_2 are the slopes of the two exponential curves and C_1 and C_2 are the intercepts on the y axis for the respective exponentials

$\Sigma\lambda_n C_n$ is the sum of all the capillary transfer rates $k_{12} + k_{13} + k_{1n}$ (16) To state the different k values individually is impossible as steady state and equilibrium conditions are not prevailing

As regards the distribution analysis in the experimental animals the radioactivity is *expressed as* a percentage of the retained *dose and on* the presumption that the specific *activity is uniform* the results represent the amount of albumin in the organs expressed as a percentage of the total remaining albumin *pool* This is not valid for the gastro-intestinal *contents* in which mainly degradation products *are present.*

For the measurements of the initial disappearance with short intervals, the values were plotted on a semilogarithmic diagram *and the slope of the* obtained curve during the first 2 hours was then determined by the

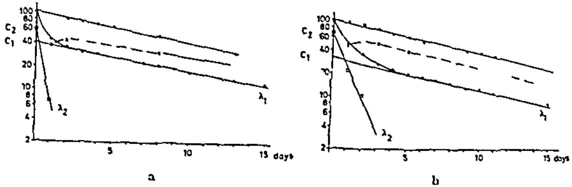


Fig 1 Plasma activity and retained dose (percent of initial values) in 2 control rabbits (nos. 2 (a) and 5b (b) given homologous ^{131}I albumin λ_1 and λ_2 denote *the slopes of the first and second exponentials*, and C_1 and C_2 the respective intercepts on the y-axis, ●—● plasma activity, ○—○ retained dose, ×—× extravascular activity For values of λ_1 and λ_2 see table VI

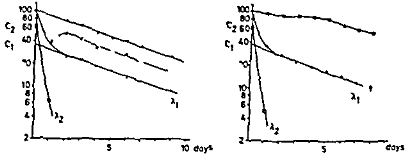


Fig 2 Two rabbits (nos 11 and 10) irradiated with 800r The curves show a steeper fall than in the controls Rabbit no 10 died on the 8th day Some deviation between plasma activity and *retained dose curves indicates extravascular retention of radioactivity Symbols as in fig 1 For* values of λ_1 and λ_2 see table VI

method of least squares ($\lambda_{t=0-2}$) The same procedure was used in calculating the *disappearance during the next 6 hours* ($\lambda_{t=2-8}$)

Results

Albumin

Data for the controls are shown in table I The plasma volumes varied between 30 8 and 44 0 ml/kg and the intravascular albumin was 1 40—1 85 g/kg The breakdown rate averaged 21—23 % of the intravascular pool In animal no 5, two examinations were made with essentially *identical results*

The two methods of determination, Campbell's and Matthews', showed fairly good agreement Expressed quantitatively, the degradation rate was 0 23—0 44 g/kg/day (table I) The serum-albumin concentration remained unchanged during the experimental periods

Judging from the graphical curves (fig 1), intra- and extra vascular equilibrium would be attained around day 3, thereafter the curves for retained dose and for intravascular activity run parallel or almost parallel The ratio of extravascular/intravascular albumin was 1 3—*2 in the controls*

TABLE II Metabolic data on the irradiated rabbits Intravascular albumin values are initial ones Figures for degradation enclosed within quotation marks are uncertain Extent of degradation was determined from initial albumin values In the 1200—2500 r group de gradation could not be determined

Rabbit no	Radiation dose (r)	Weight (kg)	P V	P V /kg	Intravascular albumin (total g)	Intravascular albumin (g/kg)	Degradation in % of i v pool: Campbell	Degradation in % of i v pool: Matthews	Degradation g	Degradation g/kg
6	275	4.45	123	27.7	5.8	1.30	27.6	27.5	1.60	0.36
7	340	4.60	128	27.8	6.0	1.30	23.8	20.0	1.20—1.47	0.26—0.32
8	400	2.25	93	41.3	4.0	1.78	26.6	22.6	0.91—1.07	0.40—0.47
9	650	2.50	104	41.6	4.9	1.96	28.9	24.5	1.20—1.42	0.48—0.57
10	800	2.22	104	46.9	3.8	1.71	24.8	40.3	"0.94 —1.53	0.42—0.69
11	800	3.13	132	42.2	5.3	1.69	44.5	37.6	1.99—2.36	0.64—0.75
12	800	3.31	111	33.7	4.9	1.48	59.0	46.6	2.28— 2.89	0.69—0.87
13	800	4.24	146	34.4	7.0	1.65	40.8	34.5	2.41— 2.85	0.57—0.67
14	865	2.50	122	48.7	5.7	2.28	27.6	23.0	1.31—1.57	0.52—0.63
15	1 200	2.50	127	50.8	6.0	2.40	Non steady state	Non steady state	—	—
16	1 200	3.06	170	55.6	6.5	2.12			—	—
17	1 600	2.50	107	42.6	5.0	2.0			—	—
18	1 600	3.79	141	37.2	6.1	1.61			—	—
19	2 500	2.41	80	33.0	4.9	2.03			—	—

In the irradiated animals (table II) the degradation as a percentage of the intravascular pool is virtually normal after the lower radiation doses but shows a rise after the 800r dose (fig 2) In terms of g/kg day the degradation is distinctly elevated at least twofold in the 800r group (table II) In calculations based on the specific activity of plasma values of the same magnitude as those in table II were obtained The urinary excretion of radioactivity was on the whole higher in the irradiated animals than in the controls After the highest doses however there is some retention (table III) A very moderate increase of faecal radioactivity was noted in the irradiated animals particularly those which received the lowest radiation doses (table III)

For rabbits nos 11 and 13, in which urine production was discontinuous and collection uncertain the degradation was calculated by means of the 24 hour differences in whole body activity In rabbit no 12 the urine production was extremely uneven, with oliguria for the first 48 hours, which resulted in an unreliable value by the urinary clearance method (59 0 %) In no 10 there was a considerable divergence between the retained dose and the plasma activity curves (fig 2 right), a phenomenon which was common to all the animals that received 1200r or more (fig 4) This leads to a clear discrepancy in catabolic

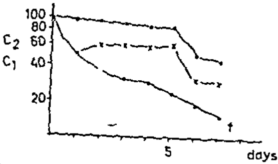


Fig 3 Rabbit no 15 irradiated with 1 200r *A sudden release of radioactivity occurs on day 6 which may indicate some restoration of intestinal absorption capacity The animal died on day 8 however*

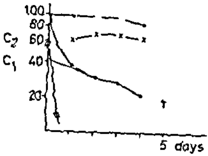


Fig 4 Rabbit no 18 irradiated with 1,600r Rapid fall of plasma activity Divergence in the curves

Table III Excretion of radioactivity (percent of dose) in urine and faeces of irradiated animals Figures in parentheses refer to days of collection

Rabbit no	Radiation dose (r)	Excreted activity in Urine	Faeces
6	275	63 (11)	1 03 (11)
7	340	62 (11)	1 80 (11)
8	400	79 (10)	0 17 (10)
9	650	70 (11)	0 17 (11)
10	800	71 (10)	0 17 (10)
11	800	75 (9)	0 05 (9)
12	800	38 (8)	0 26 (8)
13	800	46 (10)	–
14	865	70 (11)	0 45 (11)
15	1 200	61 (7)	0 17 (7)
16	1,200	12 (6)	0 15 (6)
17	1,600	55 (4)	0 20 (4)
18	1,600	17 (5)	0 11 (5)
19	2 500	14 (5)	0 18 (5)
Controls (n ~ 6)		33–60 (11)	0 05–0 18 (11)

values between the methods used (table *II*) Thus, after doses of 1200r or higher, there occurred in all the animals a non-steady state which made it impossible to calculate the catabolism by the two methods As regards the plasma activity, on the other hand, the values on days 4—5 (all the animals died on days 5—7) were distinctly lower than in the controls

Rabbit no 15 (fig 3), despite satisfactory diuresis, showed on the first 5 days a retention of radioactivity, followed on day 6 by a sudden excretion of > 45 % of the dose

In 13 animals which received 800r, the changes in the relative albumin concentration of the blood were examined (table IV) The decrease reached a maximum during days 2—5 The difference between the initial and the final values was highly significant ($p < 0\ 001$) Total protein concentration, on the other hand, was constant at 6 5 and 6 4 g/100 ml, respectively

In 8 of these animals, the absolute amount of intravascular albumin could be determined both before and 3—8 days after the irradiation (800r) The decrease is expressed as a percentage of the initial value (fig 6) The difference was significant ($0\ 05 > p > 0\ 01$)

Plasma volumes

The results of the repeated determinations of plasma volumes in the 8 animals

TABLE IV Total protein and albumin in g per 100 ml before and 4 days or more after irradiation (mean ± S D) The decrease of albumin in the 800r group is highly significant

Radiation dose (r)	No of rabbits	Pre irradiation		Post irradiation	
		Total protein	Albumin	Total protein	Albumin
800	13	6 51	4 09±0 33	6 46	3 31±0 38
1 200	1	6 20	3 82	6 60	3 56
1 600	1	6 80	4 37	6 80	3 60

which received 800r and the 7 animals in the 1200r group are shown in fig 5 There was good agreement between the values measured centrally and peripherally In a few animals the differences in the values between arterial and peripheral venous blood amounted to 10 %

The results show no reduction of the plasma volume In 3 animals no reduction was seen 2—12 hours before death In most cases the plasma volume increased by 10—30 % (fig 5) The maximum increase seems to occur within 6 days of irradiation

In most of the animals that survived the first 5 post irradiation days there was a decrease of venous haematocrit This reduction represented between 20 and 50 % of the initial value Calculation of the blood volume from these values was not made for reasons stated earlier

Distribution of albumin

It will be seen from table V that the amount of radioactivity in the gastro-intestinal tract after radiation doses LD_{99} is increased notably after 1 200r or more This increase is particularly marked in the gastro-intestinal contents, in which are found 7—37 % of the activity remaining in the body, as against ~ 2 3 % normally In the gastro-intestinal walls there is no very noticeable difference from the normal amounts An increase of radioactivity is also noted in the lungs The increase is to some degree proportional to the radiation dose as after 1,200r the amount of radioactivity is twice and after 1 600—2 500r three to four times as great as normally In other organs there is no detectable difference from the normal

Initial disappearance — extravascular transfer

Graphical analysis of the plasma activity curves in the metabolic experiments (figs 1—4) yields two exponential lines These are characterised by the expressions $C_1e^{-\lambda_1 t}$ and $C_2e^{-\lambda_2 t}$ In the controls the means for λ_1 and λ_2 are 0 088 and 1 77, respectively These values rise gradually with increasing radiation doses and are 0 167 and 2 75 in the group receiving 1 200—2 500r (table VI)

Since the sum of all the capillary transfer rates and the catabolic rate is $\lambda_1C_1 + \lambda_2C_2$ at t = 0 (16) the numerical value (mean) obtained in the controls

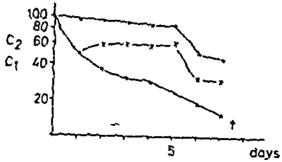


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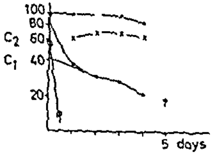


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13	800	46 (10)	—
14	865	70 (11)	0 45 (11)
15	1 200	61 (7)	0 17 (7)
16	1 200	12 (6)	0 15 (6)
17	1 600	55 (4)	0 20 (4)
18	1,600	17 (5)	0 11 (5)
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Rabbit no 15 (fig 3), despite satisfactory diuresis, showed on the first 5 days a retention of radioactivity, followed on day 6 by a sudden excretion of > 15 % of the dose

In 13 animals which received 800r, the changes in the relative albumin concentration of the blood were examined (table IV) The decrease reached a maximum during days 2—5 The difference between the initial and the final values was highly significant ($p < 0.001$) Total protein concentration, on the other hand, was constant at 6 5 and 6 4 g/100 ml, respectively

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Distribution of albumin

It will be seen from table V that the amount of radioactivity in the gastro-*intestinal* tract after radiation doses > LD_{50} is increased notably after 1 200r or more *This increase is* particularly marked in the gastro-intestinal contents, in which are found 7—37 % of the activity remaining in the body, as against ~ 2 3 % normally In the gastro intestinal walls *there is no very* noticeable difference from the *normal* amounts An increase of radioactivity is also noted in the lungs The increase is to some degree proportional to the radiation dose as after 1 200r the amount of radioactivity is twice and after 1,600—2 500r three to four times as great as normally In other organs there is no detectable difference from the normal

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Since the sum of all the capillary transfer rates and the catabolic rate is $\lambda_1 C_1 + \lambda_2 C_2$ at $t = 0$ (16) the numerical value (mean) obtained in the *controls*

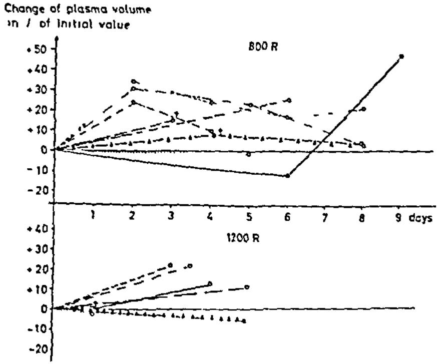


Fig. 5. Changes in plasma volume after irradiation, expressed as a percentage of initial value.

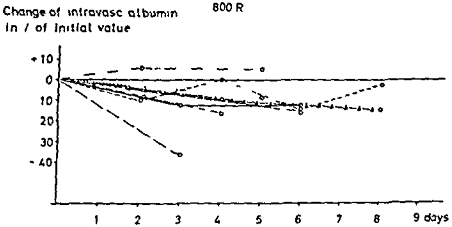


Fig. 6. Changes in intravascular albumin pool after irradiation (800r), expressed as a percentage of initial value.

is 1.12. This mean also increases with increasing radiation doses (table VI), to a maximum of 1.63.

With shorter sampling intervals, however, quite different transfer-rate values are obtained. It will be seen from the curves in fig. 7, which represent one control and one 1,200r animal, that the slope during the first 2 hours ($\lambda_{t=0-2}$) is about three times as steep as that calculated with larger sampling intervals (table VII). This is also valid for the irradiated animals, as will be seen from the table, but the difference

TABLE V Distribution of ^{131}I albumin in organs after different irradiation doses The figures denote the activities present as percentage of retained doses Figures in parentheses refer to days between irradiation and autopsy or for controls between injection of label and autopsy

Irradiated rabbits

Organ	Controls (4—7) Means of 3 rabbits	800r (4)	800r (7)	800r (8)	1 200r (6)	1 200r (8)	1 600r (4)	1,600r (6)	2 500r (5)
Liver	6.2	9.1	4.2	2.7	3.6	3.7	3.7	9.6	5.5
Thyroid	0.3	0.1	0.3	0.5	2.0	3.8	3.0	0.7	0.2
Heart	0.4	0.4	0.5	0.4	0.6	0.3	0.3	0.7	0.2
Muscle/g	0.03	0.02	0.02	0.03	—	0.02	0.02	0.03	0.03
Kidney	1.5	2.1	1.3	1.3	4.1	2.0	2.0	2.2	3.1
Spleen	0.03	0.05	0.05	0.02	0.03	0.03	—	0.08	0.03
Lungs	0.75	0.8	1.1	0.7	1.3	1.5	2.8	4.5	4.1
Gastro-intestinal walls	8.80	9.1	8.5	2.9	3.9	7.3	11.2	14.3	8.2
Contents of gastro-intestinal tract	2.3	4.2	1.7	2.7	7.1	16.0	36.8	17.7	10.8
Total activity in gastro-intestinal tract	11.1	13.3	10.2	5.6	11.0	23.3	48.0	32.0	19.0

within the highest irradiation group is not so great The mean $\lambda_{t=0-2}$ is equal in the controls and the 800r group ($\sim$3.8) but in the 1 200r group it is $\sim$ 3.0

The slope for the next 5—6 hours ($\lambda_{t=2-8}$) determined at shorter intervals is numerically well consistent with $\Sigma\lambda_n C_n$ *derived from the metabolic curves* This is valid both for the controls and for the irradiated animals as will be seen from a comparison between tables VI and VII

Discussion

Since the gastro-intestinal tract is one of the more radiosensitive organ systems (8) with rapid destruction of the epithelium and of characteristic cell features, particularly noticeable on the 3rd—9th post irradiation days (1) and since quantitatively this organ system plays an important part in the catabolism of serum albumin (27), it is reasonable to look for a relationship between the post irradiation hypoalbuminaemia and the gastro-intestinal damage Palmer and Sullivan (22), in 1959 showed that both whole body irradiation and intestinal irradiation alone induced the same degree of hypoalbuminaemia with a maximum on day 6 These authors suggested that the cause was either a direct effect on the intestine or an indirect effect on the liver with inhibition of the synthesis Sullivan (21) and Barandun (3) noted an increased intestinal loss of ^{131}I PVP with a maximum on the 4th post irra

TABLE VI Values for λ_1, λ_2 and $\Sigma \lambda_n C_n$ $_{(n=2)}$ in controls and irradiated rabbits. Columns on the right show mean and ranges

Radiation dose (r)	Rabbit no	λ_1	λ_2	$\Sigma \lambda_n C_n$	λ_1 mean, range	λ_2 mean, range	$\Sigma \lambda_n C_n$ mean range
Controls	1	0 0592	1 61	1 051	0 088,	1 77	1 19
	2	0 0815	2 31	1 417	0 059—	1 02—	0 73—
	3	0 0755	2 56	1 492	0 114	2 56	1 49
	4	0 114	"3 46	"2 02			
	5a	0 107	1 38	0 91			
	5b	0 089	1 02	0 73			
275—400	6	0 115	1 82	1 174	0 102	2 30	1 38
	7	0 0835	1 92	1 203	0 084—	1 82—	1 17—
	8	0 108	3 15	1 750	0 115	3 15	1 75
800—865	10	0 165	2 66	1 740	0 158,	2 33	1 46
	11	0 147	2 31	1 551	0 114—	1 65—	1 094—
	12	0 213	1 82	1 211	0 213	3 20	1 740
	13	0 147	1 65	1 094			
	14	0 114	3 20	1 715			
1 200—2 500	15	—	—	—	0 167,	2 75,	1 63,
	16	0 173	2 77	1 523	0 136—	2 31—	1 48—
	17	0 182	3 15	1 841	0 182	3 15	1 84
	18	0 178	2 77	1 685			
	19	0 136	2 31	1 482			

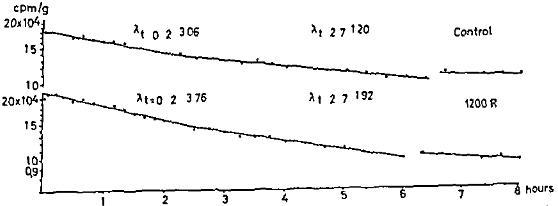


Fig 7 Disappearance of [131]I albumin from circulation during the first 2 hours ($\lambda_{t=0-2}$) and the ensuing 6 hours ($\lambda_{t=2-6}$) in one control and in another rabbit 4 days after 1 200r irradiation

diation day Birke et al (4) showed also that albumin catabolism is raised after whole body irradiation

The present study shows a distinct percentage increase and an absolute increase of albumin catabolism at LD_{50}. After lower radiation doses no significant rise was demonstrated in rabbits, but there was a moderately increased faecal activity suggesting some intestinal damage although it was not sufficient to produce a metabolic response

Parallel to the increased catabolism there is a significant reduction of the relative albumin concentration The absolute amount of intravascular albumin is also reduced though not to the same extent because an increase of the plasma volume occurs Whether hypoalbuminaemia also developed at the 400r level was not investigated here, but the normal catabolism argues against the development of any noteworthy degree of hypoalbuminaemia at half LD_{50}. The total protein content remained unchanged because of a rise in globulins Unchanged total protein has also been observed by Ditzel (11)

Two disturbances began to appear at 800r (LD_{50}) and were quite distinct at 1 200r namely irregular or reduced urinary output and retention of radioactivity These disturbances make it impossible to obtain an accurate measure of the catabolism of albumin

That retention of radioactivity occurs is evident from the marked divergence between the plasma activity and the whole body activity curves Is this an extravascular retention of ^{131}I albumin or of degradation products (^{131}I iodide)? The course in rabbit no 15 and the

TABLE VII $\lambda_{t=0-2}$ represents the initial disappearance and $\lambda_{t=2-8}$ the subsequent phase

Radiation dose (r)	Interval (hours) between irradiation and investigation	$\lambda_{t=0-2}$	$\lambda_{t=2-8}$
Controls		4 75	—
		4 72	0 85
		3 06	1 20
		3 06	0 93
		4 81	1 30
		2 58	1 61
Mean		3 83	1 18
800	24 h	3 53	—
	72 h	3 02	1 21
	72 h	3 38	1 81
	120 h	5.51	—
	192 h	3 16	—
Mean		3 72	1 52
1 200	24 h	2 41	—
	24 h	2 81	1 25
	84 h	2 42	1 52
	96 h	3 38	—
	96 h	3 76	1 92
Mean		2 96	1 56

distribution studies provide part of the answer to this question The former shows that degradation products are retained and that in some cases they can be suddenly released This may signify a restoration of the absorption ability of the intestine That the excretion of ^{131}I iodide is reduced after ionising radiation despite continued diuresis has been shown by Storer and Simonsen (23) and Ulmer et al (26) The distribution studies demonstrate that the retention is localised mainly

to the gastro intestinal contents The last-named authors, in their investigations into the distribution of ^{131}I iodide after ionising radiation in rats, found high amounts of iodide in gastric contents but very low amounts in intestinal contents, in contrast with the findings in the controls They considered this to indicate a lowered intestinal ability to excrete iodide after irradiation Such a reduction lends strong support to the assumption that there occurs a pathological loss of ^{131}I-albumin, but not of ^{131}I iodide, into the intestinal tract where the labelled albumin is then broken down Absorption of the degradation products with accompanying renal excretion is possible after doses $\leqslant LD_{50}$, whereas higher doses lead to reduced absorption accompanied by retention but also to disturbed diuresis That the intestinal absorption capacity is reduced has been shown by Detrick et al (10), and Sullivan (25)

Next in order of radiosensitivity is the capillary network of the lungs in which four times the normal activity is found after high doses of ionising radiation No studies were made here to find out how large a part of this amount was referable to plasma-^{131}I-albumin and extravascular ^{131}I albumin The lungs were by then moderately oedematous with subpleural haemorrhages

The relatively insignificant increase of faecal radioactivity in the animals that received > 400r is in all probablity attributable to a greatly impaired intestinal motility, a phenomenon that has also been noted by Conard (6) and was also seen in our experiments, where the intestine was flaccid and paralytic

Plasma volumes

Lukin (14) and Lukin and Gregersen (15) showed that the plasma volume kept fairly constant or even increased slightly up to 10 hours before death (80—100 hours after irradiation) in dogs that received radiation doses of $3 \times LD_{50}$ At the said time the plasma volume decreased by 35—48 % Three of the rabbits that received 1—1 5 × LD_{50} and died 2—12 hours after the plasma-volume determination did not show any such decrease In rabbits, the plasma volume is throughout well maintained or increased The results were identical whether the samples were taken centrally or peripherally The maintenance or the increase of the plasma volume is in all probability compensatory, as the red cell volume undergoes a distinct reduction The latter begins as early as the 2nd post-irradiation day (14, 15) There is no correlation between this decrease and the haematocrit (12) The 20—50 % reduction of the initial value that occurred in the haematocrits is evidence of severe changes in the red cell volume, but can hardly be used with great reliability in calculation of the blood volume

The mechanism of the maintenance of the plasma volume has not been made clear, but the compensation occurs probably at the cost of the extracellular fluid, which contributes to the progressive dehydration

The retention of albumin and its degradation products in some capillary systems with accompanying hypoalbuminaemia, and the simultaneous mobilisation of extravascular fluid to the

intravascular space, are two exactly opposite processes. An explanation of this is not readily found. Elucidation could possibly be obtained by studying capillary permeability and its changes after irradiation.

Initial disappearance

Increased capillary permeability with accelerated disappearance of high molecular substances to the extravascular space has been mentioned as a factor in the acute radiation syndrome (12). These authors studied the syndrome by means of T 1824 and pointed out the presence of red cells in the lymph as a sign of pathological permeability of the capillaries.

Neumayr and Thurnher (20) noted increased protein concentration in capillary filtrates after local X ray treatment in man.

Milstein et al. (18) did not find an increased elimination of dextran (molecular weight $10—170 \times 10^3$) from the blood in dogs 24 hours after whole body irradiation with 100—400r.

Graphical analysis of the metabolic elimination curves shows a rise of λ_1 and λ_2 on comparison between irradiated animals and controls. By calculation of the sum of the capillary transfer rates and the degradation rate ($\Sigma\lambda_n C_n$) a rising tendency is found in the irradiation group.

Determinations of the initial disappearance rate ($\lambda_{t=0-2}$) at shorter intervals between the samplings show two things, namely that normally this initial disappearance rate is much higher than that derived from the metabolic curves with sampling at longer intervals ($\Sigma\lambda_n C_n$), and that there is no difference between the controls and the irradiated animals with respect to the initial disappearance rate ($\lambda_{t=0-2}$) irrespective of radiation dose and length of post irradiation interval (>24 hr). However, there was a fairly wide scattering of the values (table VII).

Disappearance measured during the first two hours after injection of ^{131}I-albumin can be regarded as representative of the capillary transfer rates plus the degradation rate. Some of the activity appearing initially in the extravascular space is redistributed to the intravascular space. In view of the disproportion between intravascular and extravascular activity in the first hour the latter can, from a practical viewpoint, be neglected and the slope during this period of time be considered to represent the true disappearance rate. This will be valid as long as the disappearance curve is a straight line.

The first very rapid phase will not be seen in studies with long *sampling* intervals. This is evident from, for instance, the fact that $\lambda_{t=2-8}$ is of the same order of magnitude as the calculated $\Sigma\lambda_n C_n$.

The initial disappearance with $\lambda_{t=0-2} =$ 3.83 as an average means that albumin leaves the circulation at a rate equal to about 4 times the intravascular pool per day. Under steady state conditions albumin enters the circulation at an equivalent rate.

It is obvious that small changes in this rate, not significantly measurable by current methods, have a very marked influence on the daily amount of albumin leaving the circulation.

The interpretation of the results obtained concerning the initial disappearance rates is therefore difficult 24 hours or more after irradiation there seems not to be any detectable increase of the capillary transfer, which finding contrasts to the figures obtained from the metabolic studies As the fastest transfer processes are not detectable by the metabolic method with longer sampling intervals, there may be other disturbances responsible, such as a decreased reentry of protein into the circulation An indication that this may be a possible explanation is the equality in the changes of $\Sigma \lambda_n C_n$ and $\lambda_{t=2-8}$ with tendencies to increase with high radiation doses Similar findings as to the $\lambda_{t=0-2}$ and $\lambda_{t=2-8}$ are made in experimental intestinal obstruction (29)

However, many aspects of the initial disappearance, methodological as well as physiological remain to be elucidated This applies not only to pathological but also to normal conditions For instance one integrating factor not covered in this paper is the influence of the circulatory status on the capillary transfer

Investigations along these lines are in progress

The influence of the demonstrated radiation induced hypoalbuminaemia on mortality from the acute radiation syndrome is difficult to assess, as this syndrome is extremely complex Undoubtedly the hypoalbuminaemia per se plays some part, but is most properly to be regarded as a manifestation of one of the pathological processes that are the cause of the lethal outcome

The increased catabolism after LD_{40} should thus be viewed as a symptom of intestinal damage which, clinically, is evidenced by many other phenomena After higher doses the intestinal damage is further accentuated, the capillary system of the lungs, for instance, is also affected, and a general metabolic non steady state is present The plasma volumes are well preserved, even compensatorily increased, but there is a decrease of the colloid osmotically active part The cellular elements of the blood are also affected

Summary

The effect of varying doses of ionising radiation on the catabolism and distribution of serum albumin has been studied in rabbits by means of homologous ^{131}I-labelled albumin

In irradiated rabbits the plasma volume seems to remain unchanged throughout or to increase The red cell volume was not determined, but markedly reduced haematocrit values indicate that it is distinctly reduced

An outspoken relative hypoalbuminaemia is induced by radiation, while the decrease of the intravascular albumin pool is less marked because of the increase of the plasma volume

The total protein concentration is unchanged

A gradual rise of albumin catabolism is seen after radiation doses up to and including LD_{40} With higher doses an accentuated non steady state occurs, which makes it impossible to estimate the catabolism by current methods The ^{131}I albumin disappears more rapidly from the bloodstream, however

After radiation doses of $> LD_{50}$ this non steady state is further developed and extravascular retention occurs of ^{131}I albumin and its degradation products A considerable retention of activity is noted in the small intestine, which signifies an increased loss of ^{131}I albumin into the intestine as well as a deficient absorption of the degradation products This points to the central role of the intestinal damage in the acute radiation syndrome The capillary system affected next in order of extent is that of the lungs, where the retention is proportional to the radiation dose

It seems that a fundamental disturbance induced by the radiation would not be only an increased capillary permeability postulated by others but possibly also a disturbed inflow into the circulation

Acknowledgement

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Catabolism and Distribution of Serum-albumin in the Dog

An Experimental Study with Homologous ^{131}I albumin

By

JARL WETTERFORS

Several studies of the catabolism of serum albumin in man and in small laboratory animals have been carried out with ^{131}I albumin during the past decade

In the dog however few studies have been performed with homologous preparations (3 29) Jarnum et al (10) in 1961 reported data on investigations with heterologous ^{131}I albumin

The present investigation with homologous ^{131}I albumin aims at obtaining more accurate information on the catabolism and distribution of albumin in the dog and also on the different transfer rates and their significance

Material and methods

Healthy mongrel dogs weighing 11—25 kg were used

In 6 dogs metabolic and compartmental analysis of the behaviour of albumin was made In 2 of these animals comparative studies with heterologous ^{131}I albumin (human) were carried out

In 1 dog the elimination of commercial human ^{131}I albumin (Amersham) was determined

The initial disappearance of ^{131}I albumin from the circulation was studied in 7 dogs

Lastly in 2 dogs the distribution of albumin in different organs was determined

^{131}I albumin

Homologous albumin was prepared from canine plasma by zone-electrophoresis on polyvinylchloride The albumin fraction was collected dialyzed and freeze-dried Labelling with ^{131}I was performed by McFarlane's (15) technique The product contained around 30µC mg albumin Ultracentrifugation was performed on some of the preparations Dialyses were made to determine the amount of non protein bound radioactivity

Injection sampling and measurements

All the dogs were given NaI in the drinking water or food for 2 days before and then throughout the period of investigation

The metabolic animals were kept in a special metabolic cage such that urine and faeces could be collected separately

Total protein in the plasma was determined by the biuret method and the different protein fractions by paper electrophoresis (18)

The administered doses of ^{131}I albumin ranged from 50 to 1[illegible]0 µC. They were given intravenously under light Nembutal an-

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TABLE I Main data for the metabolic dogs. (M) and (C) *denote the method used for calculating degradation* graphical analysis (Matthews) and urinary clearance (Campbell et al)

Dog	Weight (kg)	Plasma volume (ml)	(ml/kg)	Intravascular albumin (g)	(g/kg)	Degradation (M) %/day	g/day	g/kg/day	Degradation (C) %/day	g/day	g/kg/day
1	18.0	876	48.6	31	1.72	15.9	4.93	0.274	14.3	4.43	0.246
2	25.0	1,346	53.8	41.8	1.67	17.6	7.35	0.294	—	—	—
3	15.0	821	54.7	26.6	1.77	18.1	4.81	0.321	—	—	—
15	12.7	663	52.2	20.4	1.61	18.5	3.77	0.297	17.5	3.57	0.279
18	16.0	872	54.5	30.5	1.91	17.9	5.46	0.341	17.2	5.25	0.328
25	17.0	916	53.9	32.6	1.92	18.3	5.96	0.350	18.6	6.06	0.351
Mean			52.95		1.77	17.7		0.313	16.9		0.301
± S E M			±0.94		±0.05	±0.38		±0.012	±0.92		±0.024

anesthesia (< 30 mg/kg body weight) After 9 and 12 minutes, blood samples were drawn from another site The activity of the first sample or the mean of the two values was taken for determination of the plasma volume after correction by the factor 1 015 (28)

In the metabolic dogs a blood sample was drawn every 24 hours for 10—14 days Urine was collected daily in 5 of the dogs as were faeces *in 3 of them*

For study of the initial disappearance in 5 of the dogs blood sampling was done at 10, 20, 30 60, and 90 minutes after the injection of the label and thereafter at 2, 3, 4, 5 8, 12, and 24 hours In the other two dogs samples were drawn every 10 minutes for the first 3 hours thereafter every 20 minutes for 2 hours in one of them

For investigation of the distribution of ^{131}I albumin in different organs the dogs were sacrificed on the 4th and 7th day respectively Ten minutes prior to sacrifice ^{125}I labelled albumin was given intravenously in order to determine the plasma volume and the amount of plasma in different organs All organs were weighed to within 1 g The brain and the cutis were not investigated

Most samples were measured in 2 or 4 ml aliquots depending on the well crystal scintillation counter used (Tracerlab Super scaler SC 18 A or Versamatic II Scaler Spectrometer) To assess the true activity in samples smaller than 2 and 4 ml, respectively, *certain correction* factors were used The faecal specimens were measured after homogenisation Corrections were made for background activity and for radioactive decay by reference to a standard

Catabolic and transfer rates Distribution

Determination of the albumin catabolism is performed by graphical analysis of the plasma activity curves (12) and of the urinary-clearance method by Campbell et al (2) Both presuppose degradation in the intravascular pool or its immediate vicinity (fig 1)

The following symbols are used

λ_1 = Slope of the exponential part of the plasma activity curve

λ_2 (λ_3) = Slopes of the second (and third) exponentials

C_1 C_2 (and C_3) = Intercepts on the y axis for respective exponentials

k_{12} = Catabolic rate

k_{13} k_{14} = Transfer rates intra- to extra-vascular compartments

k_{31} k_{41} = Transfer rates extra- to intra-vascular compartments

The equation for the plasma activity curve is

$$Y(t) = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + C_3 e^{-\lambda_3 t} \quad (1)$$

As $k_{12} \int_0^\infty Y(t)\,dt = 1$ at $t = \infty$, integrating and inserting $t = 0$ (2)

$$\text{gives } k_{12} = \frac{1}{\frac{C_1}{\lambda_1} + \frac{C_2}{\lambda_2} + \frac{C_3}{\lambda_3}} \quad (3)$$

To achieve the best value of the slope (λ_1) of the exponential part of the curve the method of least squares is used

The extra- and intra-vascular distribution of albumin is calculated by four different methods

1 *Extrapolation* of the first exponential where the intercept C_1 gives the intravascular part at $t = 0$ (24) This method assumes homogeneous specific activity

2 *Equilibrium time method* (2) which assumes one extravascular pool The ratio of maximum extravascular activity to intravascular activity at the same time is equivalent to the ratio of extravascular to intravascular protein mass

3 *Activity distribution ratio* (12)

$$\frac{EV}{IV} = \frac{k_{12} - \lambda_1}{\lambda_1} \text{ or } \frac{EV + IV}{IV} = \frac{k_{12}}{\lambda_1}$$

This method does presuppose homogeneous specific activity and has been used for calculating extra- and intra-vascular albumin masses (1) According to Matthews (14) this method however is not suited for calculating protein masses only radioactivity ratio and from the total body activity the catabolic rate

4 *Solving the different transfer rates* the ratio extra- to intra-vascular albumin is given by the ratio $\frac{k_{13}}{k_{31}} = \frac{M_3}{M_1}$ (M = masses in compartments)

The sum of capillary transfer rates and catabolic rate $k_{12} + k_{13} + k_{14}$ is obtained by deriving eq 1 and inserting $t = 0$

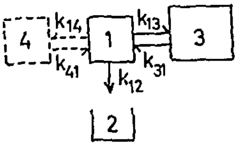


Fig 1 The compartment model used 1 = intravascular 3 and 4 = extravascular compartments. 2 is the excretory pool of degradation products Degradation is assumed to occur in 1 No intermediary pool of degradation products is introduced

$$\frac{dx(0)}{dt} = -(\lambda_1 C_1 + \lambda_2 C_2 + \lambda_3 C_3) \quad (4)$$

From the experimental determinations of the initial disappearance, mean ± S E M is derived for each sampling time This gives the slope for the first 2 hour period, during which the curve is straight Therefore that part of the curve is assumed to represent the initial disappearance (theoretically $t = 0$) The slopes for the consecutive periods 2—5 and 5—24 hrs are calculated too

Results

Human ^{131}I albumin (Amersham) was degraded very fast (fig 2) with a catabolic rate of 27.2 % ($\lambda_1 = 0.144$) In dog 1 the homologous ^{131}I albumin proved to be a good preparation with λ_1 0.0732 (fig 3 curve I) while heterologous ^{131}I albumin (human) given 3 months later showed a steep fall in the plasma activity on the 6th 7th day (curve II) During these two days > 40 % of the administered dose was excreted in the urine However, up to the 5th day the plasma curve fitted that of the homologous preparation fairly well Such a fall in the plasma content of an antigen at the end of the first

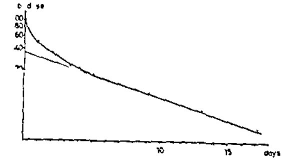


Fig 2 Degradation of commercial human [131]I albumin in the dog is fairly fast with an exponential slope (λ) 0 141 (T/2 4 8 days)

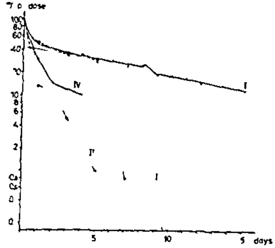


Fig 3 Degradation of homologous (•——•) and heterologous (○ ○) [131]I albumin in the dog The different curves I IV are described in detail in the text Plasma activity curves for the heterologous preparation demonstrate immune reactions (II and III)



Fig 4 The heterologous [131]I albumin shows a steeper slope initially as well as on the 5—7th days than the homologous preparation

week is interpreted as an immunological reaction After another month a second injection of human [131]I albumin was given A very fast elimination of the labelled protein occurred indicating an immediate immunological response (curve III) A few weeks later another study with homologous [131]I albumin showed a faster elimination than in the first study (curve IV)

In dog 2 the investigation was begun with the heterologous preparation (fig 4 curve I) During the first 2 days a somewhat steeper fall of activity in plasma occurred as compared with the homologous study (curve II) Then the curves parallelled each other up to the 5th day when a break appeared on the heterologous one This break coincides with the time for expected immunological response Catabolic rate for the homologous albumin was 17 6 % per day of the intravascular pool while the corresponding value for the heterologous preparation was 23 8 % (counted on the 2 5 day part of the curve)

Ultracentrifugation did not reveal any differences in the sedimentation constants between the labelled and the native albumin No extra peak was seen (fig 5)

Plasma volumes and intravascular albumin

The plasma volume was 52 95 ± 0 94 ml/kg body weight (mean ± S E M) a value in good agreement with the mean value from a larger series (30) and also with data published by *Huggins et al (8)* who in 59 dogs had a mean value of 49 2 ± 0 26 ml/kg body weight and by Spector (21) who found 52 7 ml/kg body weight

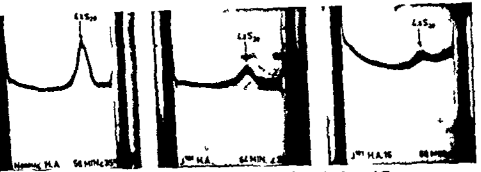


Fig 5 Ultracentrifugation of native canine albumin (left) and of two different preparations of [131]I-canine albumin

The intra vascular albumin pools were also fairly homogeneous with a mean of 1 77 ± 0 05 g/kg body weight

Catabolism of albumin

In the 4 dogs in which urinary collections were complete, less than 10 % (max 8 3 %) of the dose was excreted during the first 24 hours a fact indicating a good albumin preparation (16) During the first 5 days 30 3—34 3 % and after 10 days 53 0—53 6 % of the dose injected were found in the urine indicating a smooth and regular excretion

In the faeces insignificant amounts of radioactivity were recovered (< 0 5 %)

By graphical analysis of the plasma activity curves a degradation rate of 17 7 ± 0 38 % was achieved and by the urinary clearance method 16 9 ± 0 92 (4 dogs) Quantitatively this means 0 313 ± 0 012 and 0 301 ± 0 024 g/kg body weight respectively Thus the two methods used corresponded very well

Intra and extra vascular distribution

Estimation of the extravascular intra vascular EV/IV ratio shows good agreement between methods 2 and 3 while method 1 gives higher and method 4 lower values (table II, fig 6) Comparison of the different ratios in each dog shows grouping of the values in each individual, i e individual variations applying to all methods (fig 7) On splitting the plasma activity curves into their different exponentials it was evident that in 3 of the dogs (nos 1 2, and 25) merely two exponential terms were necessary to satisfy fully the X(t), while in the other 3 dogs a third exponential could be derived This indicates a second extravascular compartment However as will be discussed later, in 2 of the latter dogs (nos 3 18) the extra exponential is founded on very small and experimentally uncertain differences between the curve and the extrapolation line of $C_1(\lambda_1)$

Table IV shows the transfer rates between the intra and extra vascular pools achieved by compartment analysis It will be seen that the interchange between the pools ranges from 60 % to 100 % per day

The sums of the capillary transfer and catabolic rates according to the formula given under Methods are tabulated in table III, including the case of

TABLE II EV/IV ratios in the 6 metabolic dogs according to the four different methods

EV/IV ratio				
Dog	Extrapolation	Equilibrium time	Radioactivity distribution	Computed values[1]
1	1 27	1 22	1 17	1 09
2	1 03	—	0 95	0 82
3	1 78	—	1 57	1 33
15	1 27	1 26	1 11	0.96
18	1 27	1 28	1 16	1 05
25	1 56	1 40	1 39	1.23
Mean	1 37	1 29	1 22	1 07
± S E M	±0 102	±0 033	±0 09	±0 03

[1] According to the model in fig 1 and K values in table IV

TABLE III λ s C s and $\Sigma C_n\lambda_n$ for the 6 metabolic dogs These values are directly obtained by graphical analyses of the plasma activity curves

Dog	λ_1	λ_2	C_1	C_2	$\lambda_1C_1 + \lambda_2C_2$	λ_2	λ_3	C_2	C_3	$\lambda_1C_1 + \lambda_2C_2 + \lambda_3C_3$
1	0 0732	2 13	0 44	0 56	1 223	—	—	—	—	
2	0 0905	1 39	0 48	0 52	0 764	—	—	—	—	
3	0 0705	1 54	0 36	0 64	1 011	(0 478	2 56	0 13	0 51	1 397)
15	0 0877	1 39	0 44	0 56	0 816	0 866	3 01	0 32	0 24	1 039
18	0 0830	1 98	0 44	0 56	1 149	(0 301	2 77	0 10	0 46	1 342)
25	0 0767	1 65	0 39	0 61	1 040	—	—	—	—	
Mean	0 0803	1 68	0 425	0 575	[1]1 00					
± S E M	±0 0033	±0 127	±0 017	±0 017	±0 074					

[1] Or 1 038 with the value 1 039 for dog 15

a third exponential term in dog no 15 The mean value for $\Sigma C_n\lambda_n$ in the 6 dogs is 1 00 ± 0 074 The experimental figures for 7 dogs gave a mean ± S E M of 2 01 ± 0 35 (fig 8) The difference between the two means is probably significant (0 05 > p > 0 01) The connotation of this difference will be discussed later

Organ distribution

Table V shows the radioactivity in different organs and tissues expressed as fractions of the intravascular activity (= 100) As the time necessary for equilibrium has already elapsed, the specific activity may be assumed homogeneous This means that extravascular albumin with correction for the plasma

TABLE IV The catabolic (k_{11}) and transfer rates intra to extra vascular pool (k_{12}) and vice versa (k_{21})

Dog	k_{11}	k_{12}	k_{21}
1	0 159	1 07	0 98
2	0 176	0 59	0 71
3	0 181	0 83	0 60
15	0 185	0 63	0 66
18	0 179	0 97	0 92
25	0 183	0 85	0 69

content of the organs can be expressed quantitatively as well as a percentage of the intravascular pool The musculature is assumed to make up 40 % of the body weight It will thus be seen that the main part of the extravascular albumin pool is in the muscle tissue or an amount equivalent to ~ 70—80 % of the intravascular albumin The corresponding ratio for the gastrointestinal tract is 14—17 % In the parenchymatous organs the figure is only 5—6 %

Expressed in mg albumin/g tissue the highest values are found in the gastrointestinal tract with a maximum of 9—15 mg in the stomach This value may be a little too high as 2—4 % of the activity not corrected for plasma is ¹³¹I iodide (31) In the gut the specific extravascular albumin content seems to decrease slightly in the distal direction, with a minimum of 6—7 mg in the colon Among the parenchymatous organs the lungs have the largest specific content 4 4—6 7 mg while the liver and spleen contain astonishingly small amounts 0 7—2 6 mg Muscle contains 3 5—3 8 mg the heart 3 8—4 2 mg

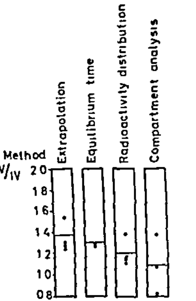


Fig 6 Distribution of the EV/IV ratios in the four different methods used The horizontal lines denote mean value for each method

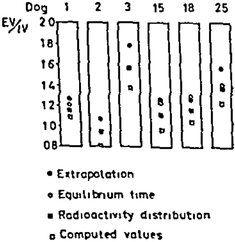


Fig 7 Distribution of the EV/IV ratios for the different methods in each dog

By the use of ¹³¹I albumin it was possible to determine the plasma content of the tissues as this protein given 10

TABLE II EV/IV ratios in the 6 metabolic dogs according to the four different methods

Dog	Extrapolation	Equilibrium time	Radioactivity distribution	Computed values[1]
EV/IV ratio				
1	1 27	1 22	1 17	1 09
2	1 03	—	0 95	0 82
3	1 78	—	1 57	1 38
15	1 27	1 26	1 11	0 96
18	1 27	1 28	1 16	1 05
25	1 56	1 40	1 39	1 23
Mean	1 37	1 29	1 22	1 09
±S E M	±0 102	±0 038	±0 09	±0 08

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1	0 0732	2 13	0 44	0 56	1 223	—	—	—	—	
2	0 0905	1 39	0 48	0 52	0 704	—	—	—	—	
3	0 0705	1 54	0 36	0 64	1 011	(0 478	2 56	0 13	0 51	1 397)
15	0 0877	1 39	0 44	0 56	0 816	0 866	3 01	0 32	0 24	1 039
18	0 0830	1 98	0 44	0 56	1 149	(0 301	2 77	0 10	0 46	1 34?)
25	0 0767	1 65	0 39	0 61	1 040	—	—	—	—	
Mean	0 0803	1 68	0 425	0 575	[1]1 00					
± S E M	±0 0033	±0 127	±0 017	±0 017	±0 074					

[1] Or 1 038 with the value 1 039 for dog 15

a third exponential term in dog no 15 The mean value for $\Sigma C_n\lambda_n$ in the 6 dogs is 1 00 ± 0 074 The experimental figures for 7 dogs gave a mean ± S E M of 2 01 ± 0 35 (fig 8) The difference between the two means is probably significant (0 05 > p > 0 01) The connotation of this difference will be discussed later

Organ distribution

Table V shows the radioactivity in different organs and tissues expressed as fractions of the intravascular activity (= 100) As the time necessary for equilibrium has already elapsed, the specific activity may be assumed homogeneous This means that extravascular albumin, with correction for the plasma

minutes prior to death is during this time distributed only in the plasma space These figures are also set out in table V The maximum values are exclusively found in the parenchymatous organs In the gastro-intestinal tract the highest amount of plasma is in the small intestine, while the stomach and the large bowel contain only about half that amount or less

It is also of interest to evaluate what proportion of the total albumin content in 1 g of wet tissue is referable to trapped intravascular albumin It will be seen from the table that in the parenchymatous organs between 50 and 90 % is plasma albumin The liver and kidneys are outstanding in this respect (70—90 %) In the gastro-intestinal tract except for the stomach and colon, the figure is about 25 %

Discussion

Homologous heterologous ^{131}I albumin

Several investigations in smaller animals (rats rabbits) have shown that the use of heterologous proteins in metabolic studies invalidates the results because of an immunological response on the 6th—7th day (4 6 11 26) This means a sudden disappearance of the actual protein from the blood because of an antigen antibody reaction

However Jarnum et al (10) in 10 dogs did not obtain immunological reactions in investigations with human ^{131}I albumin Is the immunological level of response in the dog lower than in other animals? According to the investigations presented here, it is not and it was therefore considered irrelevant to perform metabolic studies in the dog with a heterologous preparation Either a typical immunological reaction appears on the 6th—7th day or there may be an increased catabolic rate In one out of two experiments it appeared that the introduction of iodine into the protein molecule may in some way alter the immunological determinants as cross reaction occurred, indicating that antibodies against heterologous ^{131}I albumin can to some extent react with homologous ^{131}I albumin as well

Canine ^{131}I albumin containing 30 μC/mg of albumin did *not* show any change of the sedimentation constant (S_{20}) compared with native albumin, nor was there any extra peak

The low excretion of radioactivity during the first 24 hours, and the homogeneous excretion in those 4 dogs in which the urinary collections were complete and regular indicate metabolically satisfactory preparations So does also the very small S E M ($\pm$ 0 38 %) in the catabolic rate derived from graphical analysis T/2 for the exponential part of the curve *was 7 7—9 0 days This* corresponds well *with that of Deichmiller et al* (3), 8 3 days but not with Wasserman and *Mayerson s (29) value of 5 5* days However, the *intercept* C_1 *is* of importance in judging possible denaturation Low intercepts with an early high excretion of activity points to partial denaturation with rapid elimination of *the denaturated* protein The average intercept C_1 *of* 0 425 $\pm$ 0 017 does not *indicate* such a denaturation

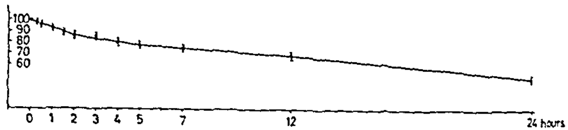


Fig 8 The initial disappearance of homologous ¹³¹I-albumin in 7 dogs. Mean and standard error of the mean is given for each sampling time for t = 0—2 h is 2 01 ? for t = 2—5 h is 0 835 and for t = 5—24 h 0 436

TABLE V Data on the distribution of extra and intra vascular albumin in different organs of 2 dogs

Organs	Weight (g)		Extravascular albumin in tissues (mg/g)		Plasma content of tissues (ml/g)		Fraction of total albumin in tissues referable to trapped plasma		Extravascular albumin in organs as per cent of i v pool		Σ %
Liver	330	263	0 7	2 0	0 15	0 14	87 7	77 1	1 2	2 1	4 8—5 9
Spleen	109	42	1 5	2 6	0 05	0 10	54 9	64 5	0 9	0 4	
Lungs	64	87	4 4	6 7	0 09	0 15	45 1	49 5	1 5	2 4	
Kidneys	61	57	2 3	3 1	0 13	0 23	70 4	77 0	0 8	0 7	
Pancreas	37	31	2 0	2 6	0 06	0 06	53 2	50 0	0 4	0 3	
Bladder (urinary)[1]	7	11	12 3	12 9	0 02	0 03	6 8	11 3	0 5	0 6	
Heart	74	78	3 8	4 2	0 04	0 05	29 1	33 9	1 5	1 1	67 6—83.5
Muscle	40% of b w		3 5	3 8	0 02	0 04	21 4	30 4	82	66 5	
Stomach	102	83	8 7	14 7	0 02	0 02	8 4	7 1	4 7	4 9	13 9—17 3
Duodenum	52	38	6 5	9 0	0 05	0 07	23 6	27 9	1 8	1 4	
Jejunum	124	111	5 6	8 0	0 04	0 07	23 9	28 2	3 7	3 6	
Ileum	131	89	6 7	7 7	0 05	0 08	23 0	31 9	4 5	2 8	
Colon	85	44	5 8	7 0	0 03	0 04	16 1	21 6	2 6	1 2	
Skin[2]									30	30	30
Plasma	470	532									116—137
I v albumin pool	18 8	24 8									
Specific albumin content (mg/ml)	40	46 6									
Specific activity (cpm/mg)	64	42									
Body weight	11 0	10 9									

[1] Obscure values

[2] According to ref 9 13 and 19

minutes prior to death is during this time distributed only in the plasma space These figures are also set out in table V The maximum values are exclusively found in the parenchymatous organs In the gastro-intestinal tract the highest amount of plasma is in the small intestine, while the stomach and the large bowel contain only about half that amount or less

It is also of interest to evaluate what proportion of the total albumin content in 1 g of wet tissue is referable to trapped intravascular albumin It will be seen from the table that in the parenchymatous organs between 50 and 90 % is plasma albumin The liver and kidneys are outstanding in this respect (70—90 %) In the gastro-intestinal tract except for the stomach and colon, the figure is about 25 %

Discussion

Homologous heterologous ^{131}I albumin

Several investigations in smaller animals (rats rabbits) have shown that the use of heterologous proteins in metabolic studies invalidates the results because of an immunological response on the 6th 7th day (4 6 11 26) This means a sudden disappearance of the actual protein from the blood because of an antigen antibody reaction

However Jarnum et al (10) in 10 dogs did not obtain immunological reactions in investigations with human ^{131}I albumin Is the immunological level of response in the dog lower than in other animals? According to the investigations presented here, it is not, and it was therefore considered irrelevant to perform metabolic studies in the dog with a heterologous preparation Either a typical immunological reaction appears on the 6th—7th day or there may be an increased catabolic rate In one out of two experiments it appeared that the introduction of iodine into the protein molecule may in some way alter the immunological determinants, as cross reaction occurred, indicating that antibodies against heterologous ^{131}I albumin can to some extent react with homologous ^{131}I albumin as well

Canine ^{131}I albumin containing 30 μC/mg of albumin did not show any change of the sedimentation constant (S_{20}) compared with native albumin nor was there any extra peak

The low excretion of radioactivity during the first 24 hours, and the homogeneous excretion in those 4 dogs in which the urinary collections were complete and regular indicate metabolically satisfactory preparations So does also the very small S E M ($\pm$ 0 38 %) in the catabolic rate derived from graphical analysis T/2 for the exponential part of the curve was 7 7—9 0 days This corresponds well with that of Deichmiller et al (3) 8 3 days but not with Wasserman and Mayerson s (29) value of 5 5 days However the intercept C_1 is of importance in judging possible denaturation Low intercepts with an early high excretion of activity points to partial denaturation with rapid elimination of the denaturated protein The average intercept C_1 of 0 425 $\pm$ 0 017 does not indicate such a denaturation

Degradation and distribution

The catabolism of 17.7 ± 0.38 % of the intravascular pool or 0.313 ± 0.012 g/kg per day, and the individual values for the 6 dogs, do not indicate any percentage decrease or absolute decrease in catabolism in the heavier dogs, as may have been expected. Probably the range of weights is too small to give a significant deviation, or the differences within a given species are too small to be detected in a limited material like this.

A homogeneous specific activity throughout the organism is an assumption which has as yet not been proved (except for the lymph). Thus, data founded on this assumption must be read with caution. According to Campbell et al. (2), this assumption is approximately true in normal subjects with a slow catabolism, but not in pathological cases with increased catabolism. In normal dogs, this assumption may thus also be approximately valid. Analysis of the plasma activity curves gives only two exponential terms, which means at least one extravascular compartment. In 3 of the dogs, however, it was possible to derive a third exponential term. In 2 of them this exponential term had a small C and λ and in the third one a small C, but large λ. These terms, at least in the first two dogs, must be judged very critically, as they are founded on few and fairly small differences between the plasma-activity curve and the extrapolated part of it. For the present it seems wiser to reckon with only one extravascular compartment in the dog. Further investigations are necessary to prove by this approach the presence of such a second extravascular compartment.

The size of the extravascular compartment related to that of the intravascular one may be estimated by the four methods indicated earlier. Beeken et al. (1) have discussed these different methods thoroughly in man. They found that the method of extrapolation gave the highest EV/IV ratio: 1.70, while the other three methods gave mean values of 1.15—1.37. Ratios in the dog were commensurable: 1.37 and 1.09—1.29. Grouping of figures from the four methods in each individual demonstrated not only methodological differences but also the presence of individual variations. Furthermore, as only two homogeneous compartments, the intravascular and the excretory ones, are available for direct measurements, the results gained will be only approximations. Definitely inaccurate is method 1, as it assumes catabolism in all compartments. Method 2 presupposes one extravascular compartment — rather an approximation. As for method 3, its real purpose is to calculate the catabolic rate from total body activity and radioactivity ratio, not protein masses (14).

There remains method 4 which, however, to be valid needs a close analysis, especially of the initial stages of the disappearance curve. This requisite is very obvious from the discrepancy between the values for initial disappearance rate calculated from graphical mathematical analysis of the curve with fairly long intervals for sampling and those determined by experimental sampling with short intervals. This discrepancy also exists in rabbits (32). As there is less than 1 % free iodine, this fast disappearance from the blood during

the first 2 hours is explicable only by the presence of one extravascular compartment close to and in rapid exchange with the capillary bed During the first 2 hours an exponentially linear fall occurs indicating a quantitatively negligible re entry of the label during this period The total capillary transfer and catabolic rate of 2 01 means that about twice the actual intravascular albumin pool leaves the circulation in 24 hours After 2 hours however the slope is less steep indicating that flowback is increasing and that the fast hypothetical compartment is in or nearly in equilibrium with the intravascular one Thereafter "slower extravascular compartments are filled up perhaps via the fast hypothetical one

This points to a certain inadequacy of metabolic studies to reveal fast processes or transfers in the vicinity of the capillary bed Thus the EV/IV ratio of 1 09 ± 0 08 may be an underestimation in a purely topographical sense but not when seen strictly functionally that is the EV IV ratio will always be an approximation

The data render it highly probable that there exist several different extravascular compartments anatomically as well as functionally undefined The derivation of a single extravascular compartment is thus an oversimplification of the distribution problem and accordingly the different transfer rates K_1, K_{II}) and so on are a resultant conection of several unknown K values

Distribution of albumin in different organs

The gastro-intestinal tract has the largest extravascular albumin pool *per g of* tissue with a maximum for the stomach the small intestine being the second in order Extravascular albumin corresponding to 14—17% of the intravascular pool is located in the gastro-intestinal tract Matthews (13) in rabbits, found 5—12 % The musculature is evidently the site of the main part of the extravascular albumin equivalent to 70—80 % of the intravascular albumin This conclusion was also reached by Matthews (13) and Oeff and Konig (17) The latter also found about 3 7 mg per g of muscle using an exsanguination technique According to Gitlin et al (7), the concentration of albumin in the interstitial fluid of muscle is 1/4—1/5 of that of plasma and the total amount about equal to that in plasma Rothschild et al (19) found that the muscle albumin accounted for 15 % of the total exchangeable albumin pool

The cutis and subcutis were not investigated but consistent results have been obtained by others Rothschild et al (19) Humphrey et al (9) and Matthews (13) stated that the skin content of extravascular albumin is ~ 30 % of the intravascular pool

Another finding is that the extravascular albumin content of the parenchymatous organs excluding *the* lungs is very small but a possible explanation will follow As the *mucosa* of the urinary bladder was not removed in the preparation a certain contamination with urinary ^{131}J iodide may be a possible explanation of the high specific value for this organ

This investigation together with those of the cutis by others demonstrate that the extravascular pool is more than

Degradation and distribution

The catabolism of 17 7 ± 0 38 % of the intravascular pool or 0 313 ± 0 012 g/kg per day, and the individual values for the 6 dogs, do not indicate any percentage decrease or absolute decrease in catabolism in the heavier dogs, as may have been expected Probably the range of weights is too small to give a significant deviation, or the differences within a given species are too small to be detected in a limited material like this

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were performed with homologous ^{131}I albumin

It is shown that heterologous ^{131}I-albumin does not give reliable results because of an immune response at the end of the first week or an increased catabolic rate

The mean catabolism is 17 7 % of the intravascular pool per day corresponding to 0 31 g per kg per day

Consideration is given to the extravascular pool and to its relative size assessed by different methods

Determinations of the different exponentials by graphical analysis do not reveal the fastest extravascular compartments close to the capillary bed

Determinations of the extravascular pool in different organs were made by a double isotope technique Quantitatively the main part of the extravascular pool is in the muscle mass The gastrointestinal tract seems to have the specifically highest extravascular content together with the lungs The maximum is in the stomach then a decrease occurs in the distal direction This pool is low in parenchymatous organs but on the other hand the latter have a high plasma albumin content which may indicate the presence of very fast compartments in close connection with the capillary bed This view is supported by the difference in the graphically and experimentally determined sum of capillary transfer rates

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100 % of the intravascular one, the subcutaneous and connective tissues not being taken into consideration

From the ^{131}I-figures the plasma content of the organs could be estimated at the moment of death The highest plasma content per g of tissue was found in the parenchymatous organs with a *maximum for the liver, lungs, and kidneys* Next came the gastro intestinal tract, where the maximum was found in the small intestine and the minimum in the stomach and colon This is in good agreement with Geber's (5) and Steiner and Mueller's (23) results from determinations of the blood-flow in different parts of the gastro-intestinal canal

The figures for *fraction of total albumin derived from trapped plasma"* are very high in the parenchymatous organs, 50—90 %, and in the gastro-intestinal tract about 24—31 %, except in the stomach This makes it probable that in these parenchymatous organs and the gastro intestinal canal, the capillary permeability is so high that even during 10 *minutes' mixing-time* so much of the labelled protein will disappear into the paracapillary space that the latter will be indistinguishable from the capillary bed This makes the plasma space" appear to be larger than it really is It was already shown by Starling (22) that the capillaries of the liver and intestine are very penetrable, since lymph leaving these organs has the highest protein content of all lymph As the determined extravascular amount of albumin in the liver is very small, *the high protein content of the lymph* may be explained simply by a more or less direct transfer *between the para* capillary space and the lymph A support for the validity of this assumption as regards the small gut as well is provided by the investigations of Swann et al (25) who in the ileum found different disappearance rates for labelled red cells and albumin, *indicating two albumin* compartments with equilibration in close connection with the capillary bed Investigations by Serrato et al (20) point to an analogy for the kidney although not so accentuated Tasker et al (27) have in in-vitro experiments demonstrated the possibility of a three-compartment system in the muscle tissue

In conclusion, these investigations together indicate that the plasma space' *determined with ^{131}I-albumin by the* dilution technique is an over estimation from a topographic-volumetric viewpoint

The values obtained reflect a functional entity, the parts of which are indistinguishable This paracapillary space (first hypothetical compartment) seems to be largest in metabolically highly active tissues, such as the liver, pancreas, kidney, and the gastro intestinal tract, which, except for the latter, have fairly *small pools of extravascular* albumin with slower rates of exchange

The results presented here do not allow any definite conclusions regarding permeability, they more likely indicate aspects of these problems hitherto not taken into closer consideration Attempts to cover these aspects are in progress at King Gustaf V Research Institute

Summary

Investigations into the catabolism and distribution of albumin in dogs

were performed with homologous ^{131}I albumin

It is shown that heterologous ^{131}I albumin does not give reliable results because of an immune response at the end of the first week or an increased catabolic rate

The mean catabolism is 17 7 % of the intravascular pool per day corresponding to 0 31 g per kg per day

Consideration is given to the extravascular pool and to its relative size assessed by different methods

Determinations of the different exponentials by graphical analysis do not reveal the fastest extravascular compartments close to the capillary bed

Determinations of the extravascular pool in different organs were made by a double isotope technique Quantitatively the main part of the extravascular pool is in the muscle mass The gastrointestinal tract seems to have the specifically highest extravascular content together with the lungs The maximum is in the stomach then a decrease occurs in the distal direction This pool is low in parenchymatous organs but on the other hand the latter have a high plasma albumin content which may indicate the presence of very fast compartments in close connection with the capillary bed This view is supported by the difference in the graphically and experimentally determined sum of capillary transfer rates

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From the Third and Fourth Departments, Aurora Hospital, Helsinki, Finland

A Follow up Study of the Poliomyelitis Patients Treated with Tracheotomy and Intermittent positive-pressure Respirators in the Years 1954—60

By

Ossi Pettay, Paavo Makimara and Britta Runeberg

In the spring of 1954 when an epidemic of poliomyelitis was expected in Finland we began at the Aurora Hospital, Helsinki, to use intermittent positive pressure (IPPB) respirators combined with tracheotomy in all poliomyelitis cases of respiratory failure. As the results were promising from the very beginning (13) this mode of treatment has been continued throughout the following years. In order to obtain a clear picture of the results a follow-up study was performed in 1962. It concerned the patients treated in the period 1954—60.

Material

During the above-mentioned years altogether 100 polio patients were treated with this type of respirator at our hospital. The [illegible] and the age and sex distribution of these patients are given in tables I and II. The majority [illegible] were 15 years of age or more. The respiratory failure was caused by paralysis of the respiratory muscles in [illegible] cases and in [illegible] cases was due to polio-encephalitis. A swallowing paralysis complicated the picture in 14 cases.

All the 100 patients were tracheotomized and only IPPB respirators (Aga Lundia, Engström) were used. The aim was to start the treatment before the appearance of hypoxia (11). In many cases, however, ventilation was already severely inadequate on admission (12).

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Results in the acute stage

The mortality after one year of observation was 16 % and after two years 18 %. Later on 8 additional patients have succumbed. Among the deceased were 7 of those already weaned from respirator aid. The prognosis for the children was much more favourable than for the adults. Of the 24 children 4 have died and 2 remained chronic respirator patients. Of the 76 adults 22 have died and 12 need still respiratory assistance.

The duration of the respirator treatment is presented in fig. 1. Approximately 5[illegible] % of those who recovered

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Material

During the above-mentioned years altogether 100 polio patients were treated with this type of respirator at our hospital. The yearly incidence and the age and sex distribution of these patients are given in tables I and II. The majority (76 %) were 15 years of age or more. The respiratory failure was caused by paralysis of the respiratory muscles in 94 cases and in 6 cases was due to polioencephalitis. A swallowing paralysis complicated the picture in 14 cases.

All the 100 patients were tracheotomized and only IPPB respirators (Aga, Lundia, Engström) were used. The aim was to start the treatment before the appearance of hypoxia (11). In many cases, however, ventilation was already severely inadequate on admission (12).

Results in the acute stage

The mortality after one year of observation was 16 % and after two years 18 %. Later on 8 additional patients have succumbed. Among the deceased were 7 of those already weaned from respirator aid. The prognosis for the children was much more favourable than for the adults. Of the 24 *children* 4 have died and 2 remained *chronic* respirator patients. Of the 76 adults 22 *have died* and 12 need still respiratory assistance.

The duration of the respirator treatment is presented in fig. 1. Approximately 75 % *of those* who recovered

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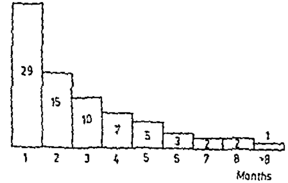


Fig 1 Duration of respirator treatment

TABLE I The yearly incidence of respirator cases

Year	No of patients
1954	33
1955	15
1956	23
1957	—
1958	4
1959	12
1960	13

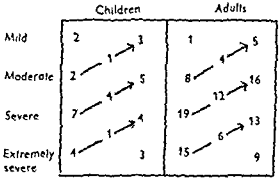


Fig 2 Alleviation of disability from an early convalescent stage to the follow up study The middle columns show the number of patients improved enough to change from one disability group to another

TABLE II Age and sex distribution of respirator cases

Age group (years)	♂	♀	Total
0–4	4	1	5
5–9	5	6	11
10–14	3	5	8
15–19	5	3	8
20–29	20	11	31
30–39	14	17	31
40–49	2	1	3
50–59	2	1	3
Total	55	45	100

sufficiently to get along without respiratory assistance did so in less than three months On the other hand, two patients became independent of the respirator only after 7 months' treatment and one after 14 months

Follow-up study

When this study began, 2 to 8 years had elapsed since the patients fell ill, the mean time interval being 5 7 years At that time 74 of the original 100 patients were alive We succeeded in examining 58 of them, 15 children and 43 adults in this study There were 14 chronic respirator patients among them

The following examinations were performed

Musculature

Evaluation of strength and function of muscles and muscle groups Following Landauer et al (6) the patients were grouped in four disability classes mildly moderately severely and extremely severely paralysed

Evaluation of the mobility of the patients

Evaluation of fitness for work

Respiratory organs

X-ray examination of the lungs

Determination of pulmonary subdivisions, maximal breathing capacity and one

TABLE III Improvement in mobility from an early convalescent stage to the follow up study

Mobility at an early convalescent stage	Mobility at the follow-up study: Immobile	Able to move in a wheelchair	Walks with crutches	Walks fairly well	Walks freely	Total
Immobile	16	4	3	3	0	26
Able to move in a wheelchair	—	5	5	2	0	12
Walks with crutches	—	—	1	3	3	7
Walks fairly well	—	—	—	—	3	3
Walks freely	—	—	—	—	10	10
Total	16	9	9	8	16	58

second forced expiration volume all with a Collins spirometer the residual volume with the helium dilution method

Determination of arterial pCO_2 with the re breathing method (2)

Urinary organs

Urine sediment glucose and protein

Plain radiography and tomography of the kidneys

The spine

In the X ray examinations deformities of the spine were also looked for

The heart and circulation were examined and the social conditions of the patients and their families were investigated These results will be published later

Results

The musculature

Fig 2 shows the grouping of the patients according to the severity of their disability at an early convalescent stage and at the follow up study Almost 60 % of the patients belonged primarily to groups severely or extremely severely paralysed Only three cases were estimated as mild They had all had respiratory paralysis of central origin due to polioencephalitis At the follow up study the percentage of patients severely and extremely severely paralysed had decreased to 50

Table III shows the improvement in the mobility of the patients during the same period as in fig 2 The proportion of totally immobile patients had diminished from 45 to 28 % and that of patients walking easily had increased from 17 to 28 %

The respiratory organs

As to the vital capacity (VC) all the chronic respirator patients had a value smaller than 30 % of the estimated norm The highest value among them was 28 % and the mean value 13 % Of the patients weaned from respirator 10 had a value between 30 and 60 % and 34 a value above 60 %

The distribution of the maximal breathing capacity (MBC) values when also expressed as percentages of the estimated norm followed very closely those of VC

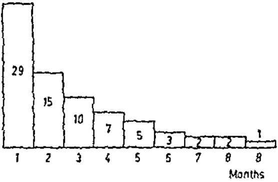


Fig 1 Duration of respirator treatment

TABLE I The yearly incidence of respirator cases

Year	No of patients
1954	33
1955	15
1956	23
1957	–
1958	4
1959	12
1960	13

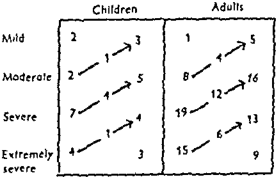


Fig 2 Alleviation of disability from an early convalescent stage to the follow up study The middle columns show the number of patients improved enough to change from one disability group to another

TABLE II Age and sex distribution of respirator cases

Age group (years)	♂	♀	Total
0– 4	4	1	5
5– 9	5	6	11
10–14	3	5	8
15–19	5	3	8
20–29	20	11	31
30–39	14	17	31
40–49	2	1	3
50–59	2	1	3
Total	55	45	100

sufficiently to get along without respiratory assistance did so in less than three months On the other hand, two patients became independent of the respirator only after 7 months' treatment and one after 14 months

Follow-up study

When this study began, 2 to 8 years had elapsed since the patients fell ill, the mean time interval being 5 7 years At that time 74 of the original 100 patients were alive We succeeded in examining 58 of them, 15 children and 43 adults, in this study There were 14 chronic respirator patients among them

The following examinations were performed

Musculature

Evaluation of strength and function of muscles and muscle groups Following Landauer et al (6) the patients were grouped in four disability classes mildly moderately severely and extremely severely paralysed

Evaluation of the mobility of the patients

Evaluation of fitness for work

Respiratory organs

X ray examination of the lungs

Determination of pulmonary subdivisions maximal breathing capacity and one-

at the extremes Another fact augmenting this tendency is that many of the patients belonging to the middle group have died They were those who succeeded only by diligent practice in getting rid of the respirator Even a small disturbance has thus been enough to bring them into a state of hypoventilation

In conformity with earlier investigations (3 9, 10), our results revealed a greatly increased RV/TC ratio of the patients lungs In respect of TC and the resting position of the lung however, our results differ from those obtained with patients treated in tank respirators If the patients with clear deformities or diaphragmatic paralysis were excluded the mean value for the TC s, which in tank treated patients was found to be normal had increased to 112 % of normal The resting expiratory position which in the tank patients was normal had clearly shifted in the inspiratory direction in our patients These changes are obviously due to an altered position of the whole thorax brought about probably by the IPPB treatment

The changes observed resemble those encountered in so-called non-obstructive emphysema This type of emphysema is presumed to be due to a weakening of the respiratory muscles and to a stiffening of the thorax (8 14) These factors are present in polio patients too The changes of non-obstructive emphysema do not impair pulmonary function but they may render the work of the respiratory muscles less economical

No severe complications arose from the tracheotomy

Summary

In the years 1954—60 altogether 100 polio patients with respiratory failure were treated at the Aurora Hospital Helsinki The majority, 76 %, were 15 years of age or more All these patients were tracheotomized and only IPPB respirators were used The mortality rate after one year of observation was 16 % and after two years 18 % A further 8 patients have subsequently succumbed There were no severe complications due to tracheotomy

In 1962 a follow up study was performed in which 58 out of the 74 surviving patients participated There were 14 chronic respirator patients among them The mean time elapsed from the onset of the illness to the follow up study was 5 7 years

In an early convalescent stage about 80 % of the patients were severely or extremely severely paralysed In the follow up study this number had decreased to 50 Sixteen of the patients were able to walk freely, but as many were totally immobile

The pulmonary subdivisions were determined in 36 cases The chronic respirator patients are not included When the patients with clear deformities of the thorax and those with diaphragmatic paralysis were excluded the total lung capacity in the rest was clearly enlarged The mean value was 112 % of the predicted normal The residual volume was enlarged both relatively and absolutely The mean value was 47 % of the total lung capacity The resting expiratory position of the lung had shifted in the inspiratory direction The forced one second expiration vol

The forced one-second expiration volume was normal, more than 80 % of the VC, in all but three cases. The p_aCO_2 was within normal limits in all patients not using a respirator. All the chronic respirator patients, however, were in a state of hypocapnia. The lowest value was 26 mm/Hg. During the years in the respirator they had become adapted to a subnormal CO_2 pressure.

The volumes of the pulmonary sub-divisions were determined in 36 patients. Owing to technical difficulties these determinations were not performed on the chronic respirator patients. The total lung capacity (TC) was less than 80 % of the predicted normal in 6 cases. The reasons for this were deformities of the thorax and spine and diaphragmatic paralysis. In 7 instances the TC was more than 120 % of the predicted normal. After exclusion of the cases in which deformities and/or diaphragmatic paralysis obviously diminished the TC, 25 patients remained. The mean value of their TC's was 112 % of the predicted normal. The duration of the respirator treatment had no correlation with the TC values.

The most striking difference from normal was found in the residual volume (RV) values. The mean of these was 47 % of TC. The highest figure was 78 %. In only 5 instances was this value within normal limits. This increase in the RV was mainly due to a decrease in the VC. This again depended mainly on a decrease in the inspiratory reserve volume. The resting expiratory level of the lungs was clearly shifted in the inspiratory direction.

Nephrocalcinosis

Forty nine patients were examined for nephrocalcinosis. This condition was found in 8 of them, i. e. in 16 %.

Scoliosis was revealed in 14 cases, i. e. in 24 %. In 5 instances the deformity was grave.

Discussion

The years 1954—60, during which the patients of this series fell ill, were among the last before the era of successful mass vaccination. As the use of the IPPB respirators developed during the same years, this method of treatment was not used to its full extent elsewhere. In the Copenhagen epidemic of 1952, during which the basic principles of IPPB respirator treatment were established (7), almost everything was still in a stage of improvisation. In Stockholm in 1953 the cuirass type respirators were more used than the IPPB ones (1). Accordingly our series is apparently the largest in which all patients with respiratory failure have been tracheotomized and only IPPB respirators used.

Our results with a mortality rate of 16 % after one year of observation and 18 % after two years, compare well with results obtained with different methods of treatment elsewhere (5, 7, 15).

The most interesting results in our follow up study concerned the respiratory organs.

As to the VC values, the smallness of the 30—60 % group is striking. It probably depends on two factors. It is known that severely paralysed muscles show a much weaker tendency towards recovery than mildly paralysed ones (4). On this account more cases tend to gather

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From the Departments of Medicine, Pori Municipal Hospital, Pori and Vasa Central Hospital, Vasa, Finland

Macroglobulinaemia Primary (Waldenström) and Symptomatic in Rheumatoid Arthritis

By

Guido Gothoni, Curt Wasastjerna and Beata Jeglinsky

According to Pedersen and Waldenström the macroglobulins are globulins with a sedimentation constant of over 15 Svedberg units corresponding to a molecular weight of over 1 million (12 20). Normally the macroglobulins constitute 1—5 per cent of the total serum proteins (4 12 20). Waldenström in 1944 was the first to describe 2 patients with a marked elevation of the macroglobulin content in serum (19). The clinical picture resembled in some respects that of multiple myeloma. Anaemia, epistaxis, slight lymphadenopathy, "lymphocytoid" cells in the bone marrow and general osteoporosis were characteristic features. In contrast, the changes of the bone system typical of myeloma were *not observable*. Chemically an increase in serum macroglobulins was demonstrable. In addition one of the patients exhibited cryoglobulinaemia. Waldenström named the syndrome macroglobulinaemia. Since the publication of his paper more than 200 cases with the same or a similar clinical picture have been described (2 from Finland (5)), and the syndrome has generally been accepted as a distinct disease, called Waldenström's macroglobulinaemia.

Later it was found, however, that elevation of the macroglobulin content in serum is a symptom of certain other diseases as well, such as nephrotic syndromes, chronic diseases of the liver, and chronic infections (8, 13, 14, 20). Hence, a secondary, symptomatic form has been distinguished from the primary macroglobulinaemia Waldenström and the macroglobulinaemia itself is consequently regarded as a symptom and not as a specific disorder.

The cause of the primary form is believed to be a proliferation of lymphocyte-like cells in the bone marrow which are regarded as the source of the pathological protein (8, 14, 20). Secondary macroglobulinaemia is, of course, much more heterogeneous, the clinical symptoms being entirely dependent on the basic disease. According to

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ume was normal in all but three cases The results obtained resemble those encountered in so called non-obstructive emphysema The arterial pCO_2 was *normal in all patients weaned from the* respirator, but subnormal in all chronic respirator patients

Nephrocalcinosis was diagnosed in 9 patients and scoliosis in 14

Acknowledgement

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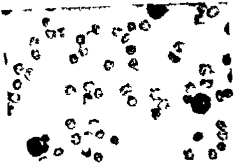


Fig 1 Case 1 Bone marrow smear 400

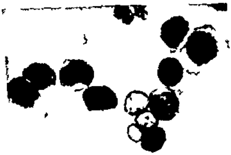


Fig 2 Case 1 Bone marrow smear 1 000

Ultracentrifugation (table 1) shows macroglobulins divided among three different fractions (S_{20} 16 7 24 2 28 0) together constituting about 40 % of the total serum proteins

Course of illness The condition has been more or less the same for over eight years There have been improvements particularly after triethylenemelamine medication for some period of time but exacerbations with pain and slight haemorrhages have also occurred After a mild trauma to the leg a haematoma developed with poor healing tendency Histologically a diagnosis of periarteritis nodosa was made Penicillamin therapy was tried and the pathological M peak in the electrophoretic pattern sank temporarily as a result of fission of the macroglobulin[2] but no definite clinical improvement resulted The bone marrow picture remained largely the same just as did the other clinical findings On the

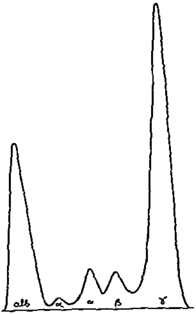


Fig 3 Case 1 Electrophoretic pattern

whole the course of the disease has been fairly benign

Case 2 A 75-year-old farmer who was admitted for the first time in January 1962 The diagnosis Waldenström s macroglobulinaemia is regarded almost certain

History On admission, he had had repeated nose bleedings for one year and periods of severe anaemia Previously he had also had rheumatic symptoms for which he had been treated at various sanatoria

Subjectively the patient experienced fatigue and weakness but no pain in the joints

Objectively the general condition was good in spite of the anaemia There was no lymphadenopathy and the heart lungs and abdomen showed nothing noteworthy The blood picture exhibited severe slightly hypochromic anaemia and leukopenia with relative lymphocytosis The Schilling test showed a B_{12} excretion of 13 3 % X ray examinations revealed no changes in the bone system. There was persistent proteinuria but not of the Bence Jones type Urinary protein was 3 4 g/l 42 % of which

Svartz and others (3, 8, 10, 16, 17, 18), the rheumatoid factor is a macroglobulin, and occasional cases have been described (8) in which rheumatoid arthritis has been associated with a marked increase of the macroglobulin content in the serum, simulating the primary form of macroglobulinaemia In other collagen diseases, too, a secondary elevation of the macroglobulin content has been observed in some cases (3, 8, 14, 20) Furthermore, in one case at least of Waldenstrom's macroglobulinaemia, the macroglobulins were found to possess properties resembling those of the rheumatoid factor in rheumatoid arthritis (7) By recent immunoelectrophoretic investigations the rheumatoid factor has been identified as a β_{2M} globulin, which fraction is markedly elevated in the primary form of macroglobulinaemia (10) A distinction between the two forms cannot be based on the results of immunoelectrophoresis alone, however The diagnosis depends on the clinical findings

The macroglobulins have been the subject of extensive laboratory studies, and most authors have concluded that the pathological protein belongs to the antibody group (1, 3, 8, 10) Many investigators tend to include these dis orders in the group of "auto immune diseases" (8, 9, 10 21)

To the best of the author s knowledge, no complete comparative clinical studies on primary and on secondary, or symp tomatic, macroglobulinaemia have been published As a rule, only serological results have been reported Since the common symptom macroglobulinaemia is probably related to other clinical features which the two forms have in common, 4 cases of macroglobulinaemia will be described, 2 of the primary, or Waldenstrom, type of macroglobulin aemia, and 2 of the secondary, symptomatic type associated with severe rheumatoid arthritis

Case reports

Case 1 A now 63 year old farmer, admitted for the first time to the Department of Medicine of Vasa Central Hospital in 1956, and since then followed up for eight years during which he has been re-admitted about ten times The diagnosis Waldenström's macroglobulinaemia is regarded as established

History Epistaxis and gingival bleeding 'rheumatoid pain" for years, weakness fatigue, high ESR value for a long time

Subjectively the patient has suffered most from the haemorrhages and from pains in different parts of the body, including the joints

Objectively the general condition is fairly good The patient is of ordinary build and the heart, lungs and abdomen show nothing noteworthy The bone system exhibits roentgenological signs of slight osteoarthritis but no myelomatous changes or signs of rheumatoid arthritis Slight hypochromic anaemia is present The ESR has varied between 98 and 150 mm/hour No lymph adenopathy is observable Sia's euglobulin test strongly positive the latex fixation test ± There has been persistent proteinuria but not of the Bence Jones type

The *bone marrow* (figs 1 and 2) exhibits over 90 % lymphocytoid cells (It proved very difficult to obtain a satisfactory bone marrow punctate, which tallies with the experience of other authors) Morphologically, these cells occupy a position between the lymphocytes and the plasma cells

Paper electrophoresis shows a typical, pointed M protein peak in the area of the γ fraction (fig 3) Total protein 12 9 g/100 ml

(degree IV) with typical roentgenological changes. There has been no lymphadenopathy and no anaemia. The spleen was not palpable. The ESR has varied between 106 and 140. The maximum Waaler Rose titre was 1 : 65 000. The CRP and latex fixation tests were markedly positive. Nucleic acid antibodies in the serum ± (1/64). A number of tests for LE cells have been negative. There has been persistent slight proteinuria. Sia's test definitely positive although not to the same degree as in case 1, the Kongo test normal. Special investigations for possible coagulation defects revealed nothing pathological.

The *bone marrow* shows an increase in plasma cells and occasional cells which can possibly be regarded as belonging to the group of lymphocytoid cells, but the finding is uncertain.

Electrophoresis shows a marked increase of the γ fraction (about 46 relative %), but the peak is not of the same "church tower shape" as in case 1, for instance; it is more extended and has a broader base, as is often the case in disorders of the connective tissue. *Total proteins 7.5 g/100 ml* (fig. 4).

Ultracentrifugation (table I). Macroglobulin content in the serum about 24 % (at a later check up about 30 %) or 1.8—2.3 g/100 ml distributed over three fractions (S_{20} 19.2, 26, 38.3).

Course of illness. The condition has remained more or less the same during the time of observation (about two years). There has been persistent joint pain and the patient is almost entirely bedridden. Occasionally she has had rather severe infections of the respiratory tract, but no haemorrhages. Repeated checks of the electrophoretic and other values have given largely the same results as the previous examinations.

Case 4. A now 56-year-old married woman who has been followed up since March 1962. Rheumatoid arthritis with secondary macroglobulinaemia is regarded as the most probable diagnosis.

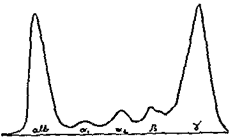


Fig. 4. Case 3. Electrophoretic pattern.

History. The patient has had joint symptoms for over thirty years, steadily exacerbating during the last few years. The finger, knee and ankle joints show marked deformation. There have been no haemorrhages nor any other noteworthy symptoms apart from the joint symptoms.

Subjectively the patient has experienced tenderness, pain and stiffness of the joints.

Objectively severe rheumatoid arthritis (degree IV) with typical deformation of the finger, knee, elbow, wrist and ankle joints is present. Roentgenologically too typical changes are demonstrable. The ESR has varied between 96 and 116 mm. Sia's test possibly positive. Waaler Rose markedly positive (with a titre of 1 : 2 000). The CRP and latex fixation tests are also positive.

The *bone marrow* exhibits only a slight increase in plasma cells, such as occurs in chronic infections. Cells of the lymphocytoid type are not observable.

Electrophoresis shows a marked increase in γ globulins (relative percentage 44), but the configuration is of the collagenous type, i.e. with a broad base and obtuse, in contrast to the sharp and narrow peaks of cases 1 and 2.

Ultracentrifugation (table I): the macroglobulins constitute about 11 % of the total serum globulins, distributed over two fractions (S_{20} 18.7, 23.5). Total protein 7.3 g/100 ml.

Course of illness. The condition has remained the same during the time of observation (about two years).

TABLE I Relative percentages of ultracentrifugal fractions

Fraction	S_{20} units	Case 1	Case 2	Case 3	Case 4	Normal
X	4		5			2 5– 5 5
A	4 0–4 7	49	41 5	57 5	63 4	85 –89
G	7 0–7 3	9	11 5	15 2	19 6	10 –13
Z	c a 10			3 6	5 6	—
	16 7	35				
M_1	18 7		39		2 9	1 – 5
	19 2			4 6		
	22 5		3			
M_2	22 6			17 7		
	23 5				8 5	
	24 2	4				
M_3	28 0	1				
	38 0			1 4		

fell within the γ range on paper electrophoresis

The *bone marrow* picture was dominated by lymphocytes, which constituted 90 % of the cells Some of them exhibited scanty or no cytoplasm, while others had a rather abundant, vacuolized cytoplasm and resembled plasma cells to some degree Many lymphocytes showed budding Mast cells were strikingly abundant

Electrophoresis showed an M protein peak in the γ fraction (albumin 45 %, α_1 4 %, α_2 7 %, β 8 %, γ 36 %, total proteins 8 0 g/100 ml)

Ultracentrifugation (table I) showed a total of 42 % macroglobulins, present in the fractions having S_{20} 18 7 and 22 5

Course of illness The severe anaemia was successfully treated by blood transfusions and with chlorambucil and prednisone The patient was discharged in good condition Somewhat more than a year after the first hospitalization he was admitted for the fourth time on account of continuous epistaxis In addition to anaemia leukopenia (about 1,000/mm³) was again present, and thrombocytopenia and a marked impairment of the general condition were noted Local treatment had no lasting effect on the nose bleeding Serum protein was now 13 g% In spite of repeated blood transfusions, corticoid and penicillamin therapy, etc., the general condition deteriorated, the temperature rose, and the patient expired with signs of sepsis and circulatory failure At autopsy nothing noteworthy was observed, apart from enlargement of the spleen (weight 450 g) and an old haemorrhage in the thyroid On histological examination the bone marrow exhibited mainly small lymphocyte like cells and plasma cells

Case 3 A now 66-year-old widow, who has been followed up at the Municipal Hospital of Pori since 1961 Rheumatoid arthritis with symptomatic macroglobulinaemia is regarded as the most probable diagnosis

History For twelve years the patient has had severe rheumatoid arthritis, gradually leading to disability There have been no haemorrhages, but infections have been relatively frequent and have aggravated the joint symptoms

Subjectively the patient has experienced pain and stiffness in various joints Foot and knee joint deformations have made her walking difficult

Objectively the clinical findings clearly reveal severe deforming rheumatoid arthritis



Fig 6 Immuno-electrophoresis with specific anti β_{2M} globulin Case 2 (above) and case 3 (below)

scope slides covered with agar gel (Ouchterlony plates) containing saline All sera were diluted 1 16 in saline since this proved to yield the most distinct bands A hole punched in the gel was charged with anti macroglobulin serum 558 H and the other sera were applied to somewhat smaller holes surrounding the central well at a distance of 5 mm from it As appears from fig 7 the antiserum reacted very weakly with normal sera and strongly with the four patient sera Complete immunological identity was demonstrable between the sera from the two patients with primary macroglobulinaemia and the two rheumatoid sera The same result although the precipitation was somewhat weaker, was obtained when the antiserum was diluted with an equal volume of saline When the antiserum was diluted with undiluted human serum no reaction ensued irrespective of whether normal serum or serum from any of the present patients was used for dilution

With the above mentioned anti β_{2M} serum and serial dilutions of the different sera the precipitin titre was determined through diffusion in agar gel as suggested by Kanzow et al (6) The following titre values were obtained Case 1

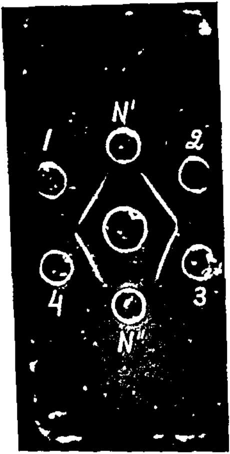
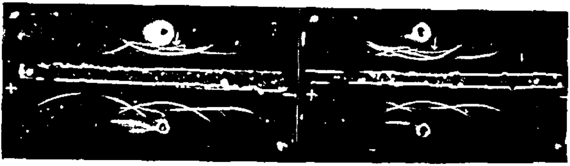


Fig 7 Immunodiffusion (Ouchterlony) Antiserum (anti macroglobulin serum 558 H) reacts faintly with normal sera (N' and N'') and strongly with the four patient sera (1—4)

1 2 560 case 2, 1 1 280 case 3, 1 640, case 4, 1 320 With four control sera from normal subjects the highest titre was 1 40

Discussion

In all 4 cases a marked increase in macroglobulin was demonstrable by ultracentrifugation and the use of the term macroglobulinaemia therefore



A B

Fig 5 Immuno electrophoresis
A Case 2 above normal serum below
B Case 3 above, normal serum below
Note heavy precipitation around the application points of sera containing macroglobulin, particularly in case 2 Arrows show the β_{2M} arcs

Immunological investigations

Immuno electrophoresis was performed on microscope slides in buffered agar-gel (pH 8 7) The antisera used were serum No 223 from the Pasteur Institute, which is a horse serum against normal human serum, and a goat serum against human β_{2M}-globulin (macroglobulin) from the Mann Research Laboratories (Cat No 558 H) After electrophoresis for four hours with a 7—8 V/cm current the fractions were allowed to diffuse for 24 hours at 37° C, after which the slides were photographed unstained in a dark field

When normal human sera were developed with antiserum No 223, β_{2M} precipitation was very indistinct or lacking Distinct and broad bands were obtained with the two rheumatoid sera In addition, a relatively strong β_{2A} reaction was obtained with serum no 4 Sera from the two patients with primary macroglobulinaemia gave a strong precipitation in the vicinity of the application points After dilution 1 4 with saline, distinct and broad precipitation bands were still obtained with the two last-mentioned sera in the area typical of the β_{2M} fraction On comparison of these bands with the corresponding bands of the two rheumatoid sera, it was found that the latter extended further towards the cathode and were thinner than the first mentioned bands (fig 5)

Antiserum 558 H (Mann) diffused against normal sera yielded a thin precipitation band in most cases and none in some Against the sera of our four patients this serum reacted in all cases with a broad precipitation band in the area typical of macroglobulin When the sera were diluted 1 4 before electrophoresis, as described above, a distinct band was again obtained with sera from the patients with primary macroglobulinaemia Again it was found that the sera of the rheumatoid patients gave longer precipitation bands than the other two sera Since only one band emerged in all cases antiserum 558 H may be regarded as a specific antimacroglobulin serum (fig 6)

Immunodiffusion tests without electrophoresis were performed on micro

stitute strong evidence in favour of primary macroglobulinaemia, but the survival time of the patient was shorter than what is usual in the primary form

Case 3, which is theoretically, perhaps, the most interesting exhibits a clinical picture of severe rheumatoid arthritis without symptoms of any concurrent disease, apart from the strikingly high macroglobulin value in the serum The macroglobulins constitute 25—30 per cent of the total serum proteins (about 1 8—2 2 g/100 ml) and are, moreover, distributed over three different fractions These findings are suggestive of the primary form of macroglobulinaemia Immuno-electrophoresis revealed an increase of the β_{2M} globulin fraction just as in cases 1 and 2 Obviously the amount of rheumatoid factor present in this case is so large that ultracentrifugation yields misleading results It thus appears that a diagnosis of Waldenstrom s macroglobulinaemia can not be based on the results of ultracentrifugation and immuno-electrophoresis alone the differential diagnosis being dependent on the clinical symptoms If for instance, a haemorrhagic tendency were present in cases 3 and 4 it would be very difficult to make a distinction between the two groups the serum protein findings being so alike In the literature at least one similar case has been reported (8) From the standpoint of differential diagnosis LED constitutes a third alternative but in the case under discussion the clinical picture and the serological results speak against this possibility although the nucleic acid antibody titre was 1 64 (±) This reaction was probably false and due to the dysproteinaemia

Case 4 exhibits the same clinical picture of severe rheumatoid arthritis as case 3, with symptomatic, relatively slight macroglobulinaemia (about 800 mg macroglobulin/100 ml of serum)

On immuno-electrophoresis using anti-human immune serum sera from patients 3 and 4 yielded a macroglobulin band with a configuration that differed somewhat from that obtained with sera from patients 1 and 2 However, immunodiffusion against a specific anti-macroglobulin serum showed indubitably that the β_{2M} fraction was immunologically identical in all four cases Furthermore, all these sera were capable of neutralizing the capacity of the antiserum to precipitate the other sera Since the same observation was made when the antiserum was diluted with an excessive volume of normal serum, it may be assumed that the pathological macroglobulins *in the present cases* are immunologically identical *with the macroglobulin that normally occurs*

Since the marked increase in the production of macroglobulin in the two primary cases obviously was related to a markedly increased proliferation of the lymphocytoid elements in the bone marrow similar findings could reasonably be expected in the secondary form too No definite pathological changes of the bone marrow and the lymphocytic system were observable however In case 3, the number of plasma cells in the bone marrow was somewhat increased but not more than in many other chronic diseases In this

TABLE II Comparison between the objective findings

	Case 1	Case 2	Case 3	Case 4
ESR (maximum)	150	179	140	116
Leucopenia	–	+	–	–
Lymphocytosis	–	++ (relat)	+– (max. 54%)	–
Thrombocythopenia	–	++ (12 000)	–	–
Prolong bleed and coag time	–	+–	–	–
Euglob (Sia test)	+++	+++	++	+–
Cryoglobulin	+–	–	–	–
Total protein	12 9 g %	10 4 g %	7 5 g %	7 3 g %
Reticulocytosis	–	+	–	–
Coombs reaction	–	–	–	–
Latex	+–	–	+++	+
Waaler Rose	<23	<45	1 65,000	>1 2 000
CRP	–	+++	++++	++
Nucleic-acid antibodies	–	–	+– (1/64)	–
LE-cells	–	–	–	–
Proteinuria	++	+	+	–
Bence Jones protein in urine	–	–	–	–

seems justified Case 1 exhibits the features typical of "classical" Waldenström's macroglobulinaemia, except that there is no enlargement of the lymph nodes, spleen and liver, as is often seen in these cases The typical changes of the bone marrow, the results of paper electrophoresis and immuno electrophoresis, etc, constitute strong evidence in favour of this diagnosis We have followed this case for eight years, and we have arrived at the conclusion that a special disorder is involved, with symptoms and a course differing from those seen in myeloma chronic lymphatic leukaemia etc Interesting in this case is the presence of pain of a typically rheumatoid" character this being the only subjective symptom apart from fatigue and a haemorrhagic tendency As a rule, this symptom has not been mentioned in the literature, the reason perhaps being that it has been regarded as insignificant In a case of concurrent cryoglobulinaemia and macroglobulinaemia the presence of rheumatic arthritis has, however, been reported (15) As compared with cases 3 and 4, the presence of pain in case 1 is of interest The histological findings in a biopsy specimen taken from the tissue surrounding a traumatic haematoma also seemed to suggest that a collagen disease" (periarteritis nodosa?) is involved However, there are no other positive objective findings pointing in this direction The latex fixation test was not definitely positive, and the Waaler-Rose titre was within the normal range

In case 2 it is difficult to exclude the possibility of lymphatic leukaemia In addition to the results of ultracentrifugation, the bone marrow findings con-

cent in the other two cases. By immunoelectrophoretic investigations the pathologically increased globulin was identified as belonging to fraction β_{2M}. Using the Ouchterlony technique it was found that the fraction was immunologically identical in the four cases. With reference to Burnet's clonal selection theory the possibility is discussed that transitional forms exist between Waldenström's macroglobulinaemia and, for instance, rheumatoid arthritis.

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connection it is interesting to note that unusually *high* titres of rheumatoid factor have been demonstrated in some cases of rheumatoid arthritis with splenomegaly (8) Furthermore, the results of certain investigations performed by an isotope technique suggest that the rheumatoid factor macroglobulin is produced by elements in the lymphocytic system (10)

The absence of a haemorrhagic tendency in the symptomatic cases seems *to indicate that this tendency in* Waldenstrom's macroglobulinaemia, noted also in the present study, is not directly secondary to the dysproteinanaemia, but is a separate, essential symptom

Chemically and immunologically, there are close resemblances between the two cases of primary macroglobulinaemia and the two cases of rheumatoid arthritis with secondary macroglobulinaemia Clinically, the two disorders as a rule have little in common, apart from the protracted course If an attempt is made to explain the relationship between the present cases by the instructive immunological theory, on the assumption that the macroglobulins are antibodies, the existence of a common antigen has to be postulated The aetiology of the disorders in question being entirely unknown, this possibility is not excluded, but then the fact that the disease pictures mostly are so different is not accounted for The rheumatoid factor is regarded by many authors (1, 3, 8, 10) as an anti antibody, i e as an antibody against 7 S γ globulin If the macroglobulins in Waldenstrom's disease also are anti-antibodies, the fundamental mechanism might be quite different causing different symptoms, and the only common feature would be the development of anti antibodies in the form of macroglobulins The formation of immunologically identical high molecular-weight globulins in two different diseases is best accounted for by Burnet's clonal selection theory Without discussing the question of whether the cause is the same in the two diseases, it may be assumed that the antibody forming tissue is changed in a similar way *in rheumatoid arthritis* and primary macroglobulinaemia

Waldenstrom (21) has shown that there is a relationship between diseases associated with hyperglobulinaemia of the M type and combinations of such disorders It is not possible to draw any far-reaching conclusions from the present few cases, but we believe that our results to some extent corroborate the hypothesis that a clinical, chemical and immunological relationship exists between disorders which have macro*globulinaemia as a common symptom* Such disorders are, for instance, primary macroglobulinaemia and certain cases of connective tissue disorders

Summary

In 4 cases macroglobulinaemia was established by ultracentrifugation of the serum Two cases were of the type called primary or Waldenstrom's macroglobulinaemia two of the symptomatic macroglobulinaemia type associated with severe rheumatoid arthritis In these cases the macroglobulins constituted about 40 per cent of the total serum protein, as compared with 25 and 11 per

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A Case of Aplastic Anemia Successfully Treated with Androgens

By

PER BJÖRNTORP, ALEKSANDER WEINFELD and PER LUNDIN

Aplastic anemia due to drug toxicity is a serious disease with a bad prognosis The fatality rate is especially high in those cases with a physiological and anatomical failure of the bone marrow and with severe reduction of all cell elements in the peripheral blood (4, 13) Although spontaneous remissions occur (1 6 15 16) they are scarce

The antibiotics have made it more practicable to combat infections and corticosteroids have proved beneficial in treating the hemorrhagic diathesis The corticosteroids may also help in treating some cases of aplastic anemia in which hemolysis is an additional factor in causing anemia (8 11) or in reducing the side reactions connected with blood transfusions There are no reports however that corticosteroids increase the cellularity of the marrow or diminish the pancytopenia (9) On the contrary some authors believe that corticosteroids increase the fat content of the bone marrow when given in large doses (13) In animal experiments corticosteroids did not show any stimulating effect upon the bone marrow (2)

A new approach to treatment of bone marrow failure was initiated by Rosenthal and Erf (12) and by Gardner and Pringle (3), who in cases of myelofibrosis with myeloid metaplasia with effectively used testosterone propionate or testosterone enanthate The stimulating effect of androgenic hormones on erythropoiesis has been shown in patients with advanced breast cancer and anemia (5 6) and in eunuchs (9) Shahidi and Diamond (13 14) were the first to use androgens in children with aplastic anemia usually with a good therapeutic effect

Reports of the effect of androgen treatment in aplastic anemia of the adult are still few however Thus Gardner and Pringle treated a case with aplastic anemia with androgens and failed to note any response (3), and Kennedy (7) got remission in only one of four cases of aplastic anemia treated

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factor and vitamin B_6 without any effect on the cell count or bone marrow histology Subcutaneous petechiae were present throughout, he had several episodes of bleedings from nose and gingiva and one short period of paradental infection successfully treated with penicillin. On March 12 1963 testosterone enanthate therapy was begun The patient was given 500 mg intramuscularly (Testoviron Depot Schering) once a week during two months then 250 mg a week until treatment was stopped At the end of May the need for blood transfusions decreased (fig 1) He got the last transfusion on June 16 At this time also some side effects of androgen treatment were noted, namely a moderate furunculosis of the face and neck increase of neck circumference and an increase of libido Glutamic pyruvic and glutamic-oxalacetic acid transaminases in serum increased slightly (58 and 105 units respectively) These values returned to normal when androgen treatment was stopped The hemoglobin concentration which was below 7 0 g/100 ml began to rise in the middle of July (fig 1) The average increase in hemoglobin concentration during the following two months was about 2 g/100 ml per month and in the second part of September the hemoglobin level reached a peak value of 12 4 g/100 ml Correspondingly the platelet count rose from about 15 000 mm³ to 70 000/mm³ while the white-cell count showed a less marked rise in the number of granulocytes The petechiae and bleedings from nose and gingiva disappeared in the middle of July and the patient now felt healthy and with good strength

On September 21 1963 the last testosterone injection was given This was followed by a rebound phenomenon with a hemoglobin concentration of 10 7 g/100 ml on November 13 with a subsequent return to a constant value of about 12 g/100 ml

The white-cell and platelet count however did not become normal The first varied between 3 000 and 4 000 mm³ with 20-30 % granulocytes and the last between 50 000 and 100 000 mm³ A new sternal biopsy (fig 3) performed in the second half of September revealed an almost fat free tissue with a cellular hyperplasia dominated by erythroblasts In some fields of vision numerous megakaryocytes were seen The differential count of the marrow smear showed a relative increase in erythropoiesis which was normo-macroblastic with slight anisocytosis The quotient of erythroblasts to immature myeloid cells was 5 7 Myelopoiesis was normal Thrombopoiesis was poorly represented in the smear preparations

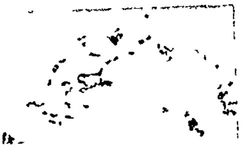


Fig 2 Histopathologic section of bone marrow before androgen treatment The specimen is dominated by fat tissue



Fig 3 Histopathologic section of bone marrow after treatment with testosterone enanthate Note the normoblastic hyperplasia and absence of fat tissue

Discussion

The pancytopenia and severe bone marrow hypoplasia encountered in this patient was probably caused by chloramphenicol as no other possible etio-

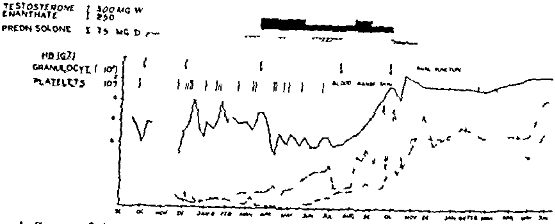


Fig 1 Survey of the hematological course and response to treatment

with androgens and corticosteroids In the following a case with aplastic anemia, probably caused by chloramphenicol, in which a remission was obtained after two months of treatment with testosterone enanthate will be reported

Case report

The patient is a 45-year old art dealer who apart from a virus hepatitis in 1945 was healthy in the past During the last three years he had *taken chloramphenicol on* repeated occasions each time less than 10 capsules of 250 mg over a period of less than one week In all he consumed approximately 100 capsules Originally it was prescribed for a gastroenteritis but it was later used for all kinds of complaints The last capsules were taken within a month before first admission In the beginning of September 1962 the patient had several attacks of nose bleeding He was admitted September 18 because of anemia On admission the patient was pale and looked acutely ill He had purpuric spots and ecchymoses in the skin and gingiva The spleen liver and lymphatic glands were not enlarged and no other abnormalities were found on physical examination Laboratory examination revealed an anemia and pancytopenia with the following values Hb 7 0 g/100 ml red cell count 2 0 mill /mm³ white-cell count 2 400/mm³ platelet count 13 000/mm³ and a total reticulocyte count of 32 000/mm³ The differential white cell count showed 21 0 % granulocytes of which 1 0 % were eosinophilic and 0 5 % basophilic and 79 0 % lymphocytes or monocytes Osmotic fragility of red corpuscles was normal Serum iron was 181 µg% Serum B₁₂ Lactobacillus casei activity in serum and whole blood liver function tests and haptoglobin concentration were all within normal limits

Histological examination of a bone marrow section revealed a scarcity of cellular elements. The specimen was dominated by fat tissue and small lymphocytes (fig 2) The differential count of a marrow smear showed a reduction and a slight shift to the left of myelopoiesis due mainly to a reduction of mature granulocytes Erythropoiesis was normoblastic Anisocytosis and an increased number of basophilic erythroblasts were present The quotient of the number of immature erythroblasts to the number of immature myeloids cells (E/M quotient) was about 3 Megakaryocytes could not be seen The patient was treated with blood transfusions and 15 mg prednisolone daily Antibiotics were given only when infection or fever was present During the following months he was frequently admitted for blood transfusions (fig 1) The peripheral blood showed no signs of remission

In January and February 1963 he was given large doses of vitamin B₁₂ citrovorum

factor and vitamin B_6 without any effect on the cell count or bone marrow histology. Subcutaneous petechiae were present throughout, he had several episodes of bleedings from nose and gingiva and one short period of paradental infection successfully treated with penicillin. On March 12, 1963 testosterone enanthate therapy was begun. The patient was given 500 mg intramuscularly (Testoviron Depot Schering) once a week during two months, then 250 mg a week until treatment was stopped. At the end of May the need for blood transfusions decreased (fig. 1). He got the last transfusion on June 16. At this time also some side effects of androgen treatment were noted, namely a moderate furunculosis of the face and neck, increase of neck circumference and an increase of libido. Glutamic pyruvic and glutamic-oxalacetic acid transaminases in serum increased slightly (58 and 105 units respectively). These values returned to normal when androgen treatment was stopped. The hemoglobin concentration, which was below 7.0 g/100 ml, began to rise in the middle of July (fig. 1). The average increase in hemoglobin concentration during the following two months was about 2 g/100 ml per month and in the second part of September the hemoglobin level reached a peak value of 12.4 g/100 ml. Correspondingly the platelet count rose from about 15 000/mm³ to 70 000/mm³ while the white-cell count showed a less marked rise in the number of granulocytes. The petechiae and bleedings from nose and gingiva disappeared in the middle of July and the patient now felt healthy and with good strength.

On September 21, 1963 the last testosterone injection was given. This was followed by a rebound phenomenon with a hemoglobin concentration of 10.7 g/100 ml on November 13, with a subsequent return to a constant value of about 12 g/100 ml.

The white-cell and platelet count, however, did not become normal. The first varied between 3 000 and 4 000/mm³ with 20-30 % granulocytes and the last between 50 000 and 100 000/mm³. A new sternal biopsy (fig. 3) performed in the second half of September revealed an almost fat free tissue with a cellular hyperplasia dominated by erythroblasts. In some fields of vision numerous megakaryocytes were seen. The differential count of the marrow smear showed a relative increase in erythropoiesis which was normo-macroblastic with slight anisocytosis. The quotient of erythroblasts to immature myeloid cells was 5.7. Myelopoiesis was normal. Thrombopoiesis was poorly represented in the smear preparations.

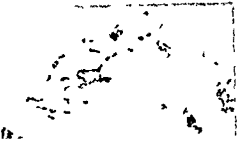


Fig. 2. Histopathologic section of bone marrow before androgen treatment. The specimen is dominated by fat tissue.



Fig. 3. Histopathologic section of bone marrow after treatment with testosterone enanthate. Note the normoblastic hyperplasia and absence of fat tissue.

Discussion

The pancytopenia and severe bone marrow hypoplasia encountered in this patient was probably caused by chloramphenicol, as no other possible etio-

logical agent was found The aplasia was *complete*, and symptoms before transfusion and corticosteroid therapy was initiated were alarming During the course of the mentioned treatment, which was continued during six months, no change occurred in the blood A sternal-marrow section showed a fatty tissue with scarce cellullarity After about two months of treatment with high doses of testosterone enanthate a diminution of the need for blood transfusion could be observed After another month of therapy, hemoglobin rose spontaneously to nearly normal values Granulocytes and platelets also increased in number but they only reached subnormal values A new sternal marrow section showed hypercellullarity dominated by normoblasts Megakaryocytes were present

Coinciding with the rise in hemoglobin concentration, side effects of androgen therapy were observed After cessation of testosterone therapy the hemoglobin level decreased for three weeks but rose subsequently to the previous level without reinstitution of therapy

The possibility of a spontaneous remission cannot be ruled out in the present case Shaw and Mc Lean (15) reported briefly the apparent recovery of a case with complete aplastic anemia due to chloramphenicol after two years of treatment with frequent blood transfusions Björkman (1) described a spontaneous recovery in a 17 year old woman after 22 months duration Such remissions are, however, rare As Moeschlin (10) pointed out they do not seem to occur earlier than after 12 -18 months' duration of the disease in severe cases

In the case reported here, the time for remission after institution of androgen therapy, coinciding with the side effects of this drug (7), and the rebound phenomenon also observed by Shahidi and Diamond (13), are in favor of a causal relationship between therapy and remission

It may be briefly mentioned here, that a good therapeutic result of androgens has been obtained in a case of completely aplastic marrow due to prolonged treatment of chronic myeloid leukemia with busulphan In another case of "idiopathic" aplastic anemia in a 75 year-old woman with rheumatoid arthritis (possible causative agent gold injections) no therapeutic effect with androgens was obtained, and the patient died of the disease The aplastic anemia in this patient was of many years' duration, and at the time of androgen therapy, which was not fully adequate in respect of dose and duration, she was in poor condition

Since androgens now seem to be the only drug which may reverse the bone-marrow failure by stimulation of erythropoiesis, these hormones should be instituted in drug-induced aplastic anemia in doses outlined by Gardner and Pringle (3), Shahidi and Diamond (13) and by Kennedy (7) In view of the severity of the disease, the side effects of androgen therapy are of little importance The therapy should begin as soon as the diagnosis has been established by histological examination of the bone marrow section and malignancy has been ruled out Androgen treatment should be supported by corticosteroids in moderate doses as to

diminish hemorrhagic diathesis and hemolysis accompanying the disease By early institution of therapy the effect of the drug might also be better evaluated, since spontaneous remission is less probable at an early stage of the disease

Summary

A 45 year old man with complete aplastic anemia, probably due to chloramphenicol, recovered after about 2 months of therapy with androgens in high doses A sustained trial of androgens, together with moderate doses of prednisolone, is recommended to be instituted at an early stage in all cases of diagnosed aplastic anemia

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Effect of Brief Carotid-sinus Pressure on Atrial and Ventricular Rhythms in Complete Heart Block[1]

By

Arne Carlsten and Folke Heyman

The atrial rhythm in cases of complete A V block often varies according to a characteristic pattern (4) the interval between two consecutive atrial contractions (P P intervals) is shortest when it includes a ventricular systole Roth and Kirsch (9) expressed this as a post systolic slowing of the auricles The first observation of this arrhythmia was made by Erlanger and Blackman 1909—1910 (6) on dogs operated on to produce A V heart block Several theories concerning its cause have been proposed (4 10 11) Erlanger and Blackman (6) themselves suggested that the arrhythmia was due to increased vagal tone after each ventricular systole and this view supported by the discovery of baroreceptor reflexes has been upheld by several other authors (7 9)

If a baroreceptor reflex induced by the ventricular systole influences the atrial rhythm in complete A V block it should be possible to imitate such a mechanism by exerting a brief pressure on the carotid sinus

Submitted for publication July 15 1964

Material and methods

Three patients with complete A V block probably all *congenital* were examined Some relevant data are given in table I

The patients were at rest in the recumbent position for about 15 minutes before the investigation started Pressure was exerted manually for about half a second via the pelotte of a standard pulse receptor placed over the right carotid sinus The pressure was recorded together with ECG on a standard direct recording electrocardiograph (Elema mingograph 42 B) and was performed in several series each consisting of 10—20 pressures at 5—15 *second intervals* Long ECG recordings were made before during and after each series

Results

Carotid sinus pressure slowed the atrial rhythm distinctly (P P intervals prolonged) and the ventricular *rhythm* to a minor degree (R R intervals prolonged) in the cases with atrial arrhythmia

[1] Part of this paper was presented at the *ninth* meeting of the Scandinavian Society for Clinical Chemistry and Clinical Physiology Sandefjord June 1963

Table I

Case No	Sex	Age	Q—S	ECG findings P P (range in sec)	R R	Remarks
1	♀	33	0 08	0 70—1 03	1 44—1 55	Slight dyspnea on exertion Systolic murmur max I_{II-III} sin X ray exam enlargement of both ventricles B P 150/90 Catheterization of right side normal result
2	♀	36	0 09	0 58—0 82	1 36—1 42	Slight dyspnea on exertion Normal auscultatory findings X ray exam slight enlargement of left ventricle B P 140/80
3	♂	35	0 11	0 90—0 98	1 52—1 62	Diabetes mellitus since 1939, retinopathy Slight dyspnea on exertion Systolic murmur max I_{II} dx X ray exam slight enlargement especially left ventricle B P 160/80 Catheterization of right side normal result

(fig 1), while no effect was seen in the case without atrial arrhythmia (case No 3)

When the pressure was applied at the beginning of a P-P interval, this interval was frequently prolonged (i e the P-wave following the pressure was retarded), when applied later in a P-P interval the effect was a prolongation of the next interval The minimum time observed for producing a retarding effect on the P-wave following the pressure was 0 20 sec in case No 1 and 0 40 sec in case No 2 as measured from the beginning of pressure to the beginning of the retarded P wave

The effect usually reached its maximum in the interval after the one during which the pressure was exerted and subsided within one or a few intervals The effect of the atrial arrhythmia was often added to that of the pressure, so that the P-P prolonging effect of the latter was less pronounced when the P P interval included a ventricular systole

A series of pressures was sometimes followed by a period with a tendency to prolongation of the P-P intervals (and in case No 2 also the R-R intervals) at about the same frequency as that during the pressures (fig 2)

Discussion and conclusion

If the post systolic slowing of the atrial rhythm in complete A-V block is due to a baroreceptor reflex, the latent period of inhibition of the S A node

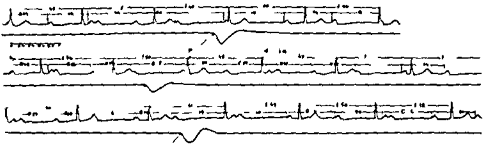


Fig 1 ECG (lead 1) from case 1 Arrows mark beginning of pressure on carotid sinus

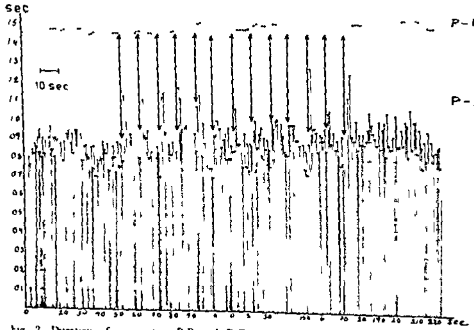


Fig 2 Duration of consecutive P P and R R intervals in case 1 Arrows mark pressure on carotid sinus

should be only a fraction of a second and the inhibitory effect should reach a maximum and begin to wane during a single heart cycle

We have seen that the minimum time for any retarding effect on the next atrial systole after pressure on the carotid sinus was no longer than a fraction of a second (0 20 sec *in* case No 1 and 0 40 sec in case No 2) These values may represent the latent period of inhibition of the S-A *node* in the respective cases They should be compared to the interval between the beginning of the systolic pressure rise in the aorta (approximately 0 10 sec

TABLE I

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1	♀	33	0 08	0 70—1 03	1 44—1 55	Slight dyspnea on exertion Systolic murmur max I_{II-III} sin X ray exam enlargement of both ventricles B P 150/90 Catheterization of right side normal result
2	♀	36	0 08	0 58—0 82	1 36—1 42	Slight dyspnea on exertion Normal auscultatory findings X ray exam. slight enlargement of left ventricle B P 140/80
3	♂	35	0 11	0 90—0 98	1 52—1 62	Diabetes mellitus since 1939, retinopathy Slight dyspnea on exertion Systolic murmur max I_{II} dx X ray exam slight enlargement especially left ventricle B P 160/80 Catheterization of right side normal result

(fig 1), while no effect was seen in the case without atrial arrhythmia (case No 3)

When the pressure was applied at the beginning of a P-P interval, this interval was frequently prolonged (i e the P-wave following the pressure was retarded), when applied later in a P-P interval the effect was a prolongation of the next interval The minimum time observed for producing a retarding effect on the P-wave following the pressure was 0 20 sec in case No 1 and 0 40 sec in case No 2 as measured from the beginning of pressure to the beginning of the retarded P-wave

The effect usually reached its maximum in the interval after the one during which the pressure was exerted and subsided within one or a few intervals The effect of the atrial arrhythmia was often added to that of the pressure, so that the P P prolonging effect of the latter was less pronounced when the P-P interval included a ventricular systole

A series of pressures was sometimes followed by a period with a tendency to prolongation of the P-P intervals (and in case No 2 also the R R intervals) at about the same frequency as that during the pressures (fig 2)

Discussion and conclusion

If the post systolic slowing of the atrial rhythm in complete A-V block is due to a baroreceptor reflex, the latent period of inhibition of the S A node

The rhythmic prolongation of the P P and R R intervals even after a series of pressures is reminiscent of an observation by Bronk et al (2) Electric stimulation of the central end of the aortic nerve or the carotid sinus nerve in cats caused rhythmic waves of activity in the sympathetic cardiac nerves After stopping the stimulation the sympathetic impulses sometimes persisted for several seconds at the same regular frequency and the centers only gradually lost the rhythm previously imposed on them

It may be concluded that the observations collected here support the view that the atrial arrhythmia in complete A V block is due to baroreceptor reflexes produced by the ventricular systole

Significance of the results

1 The sinus rhythm when slow may be steered by a feed back mechanism At each ventricular systole the vagal tone is increased and the sympathetic tone decreased Towards the end of the heart cycle this wave of inhibitory effect on the S A node passes into a wave of increased sympathetic tone and decreased vagal tone The S A node is excited a spike potential is generated and a new heart beat follows

2 There may be an oscillating tone steered by the heart beat in other parts of the autonomic nervous system As suggested previously (8) such a mechanism may be involved in the control of peripheral circulation

Summary

In cases of complete A V block there is often a characteristic atrial arrhythmia consisting of post systolic atrial slowing Brief carotid sinus pressure in two such cases produced pronounced slowing of the atrial rhythm occurring and passing rapidly like the post systolic atrial slowing On the basis of this effect on the S-A node of carotid sinus pressure, it may be presumed that the atrial arrhythmia in complete A V block is due to a baroreceptor reflex with an inhibitory effect on the S A node induced by each ventricular systole The carotid sinus pressure also slowed the ventricular rhythm, though to a minor degree

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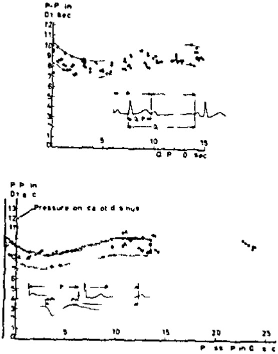


Fig 3 Upper figure Distribution of P waves and spontaneous variation of P P intervals in ventricular intervals in case 1 Lower figure Variation of P P intervals after pressure on carotid sinus Shadowed area spontaneous variation of P P intervals in upper figure placed so that the beginning of systolic pressure rise in aorta coincides with the beginning of pressure on carotid sinus See text

after Q in QRS, see fig 3) and the point in the heart cycle when the post-systolic slowing of the atrial rhythm begins (fig 3) These values were in these cases 0 30 and 0 40 sec respectively Fig 3 also shows that the slowing effect on the atrial rhythm of carotid sinus pressure and the post-systolic slowing of the atrial rhythm in the same case are similar, with a maximum about 1 1—1 4 sec after the beginning of carotid sinus pressure and also after the systolic pressure rise The "imitation" aimed at — the slowing effect on the atrial rhythm of carotid sinus pressure — thus seems similar to the "original" — the post systolic slowing of the atrial rhythm — in these cases of complete A-V heart block

It is in keeping with the above reasoning that there was no effect from carotid sinus pressure in the case of complete A-V block without atrial arrhythmia The individual variations of the baroreceptor reflex and its effect on the S-A node are probably great

Previous studies on dogs by Brown and Eccles (3) showed that the inhibitory effect of a single volley to the central end of the cut vagus slowed the heart beat maximally already in the first inhibited cycle, and that the latent period of inhibition was 0 10—0 16 sec Ashman and Gouaux (1) in similar experiments on dogs found that the latency of inhibition was 0 17 sec, and by stimuli repeated every 0 65 sec they showed that the reflex inhibition of the pace-maker waxed and waned within the time of a single cardiac cycle They concluded that their results supported the theory that the atrial arrhythmia in complete A-V block is due to a baroreceptor reflex

The effect of carotid sinus pressure in also slowing the ventricular rhythm demonstrates that the A V node is influenced by the same autonomic reflexes as influence the S A node The effect on both of these nodes may be dual i e due to both increased vagal tone and decreased sympathetic tone It would be interesting to see whether the ventricular rhythm in a complete A V block with a pace maker in the ventricles — and hence not reached by the vagus nerve — is also slowed by carotid sinus pressure

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Hypoglycemic Coma During Sulfonylurea Therapy

By

Halvar Bergman

Oral diabetic therapy has in ten years become widely spread all over the world A large material has provided frequency figures of the side effects of these agents, and it is obvious that their incidence is low and their character as a rule not particularly serious However, during the last few years an increasing number of a very serious complication — the hypoglycemic coma — has been reported (2 3—7 9—11 13 14 17 19 20, 22—29 32 33 36 38—40 41 46 49) Three such cases — two of them with fatal outcome — have been observed at Borås Hospital over a few months It has therefore seemed to be of value to review the literature in order to analyze *firstly* such factors which may contribute to this kind of hypoglycemia and *secondly* characteristic features if any of this coma In addition some practical conclusions have been drawn about the prevention the diagnosis and the treatment of the coma

The oral hypoglycemic agents that will be discussed are all sulfonylurea compounds chlorpropamide tolbutamide, carbutamide and metahexamide) In one of the cases to be reported tolbutamide and biguanide were given simultaneously However, since there has been no report in the literature on hypoglycemic coma following upon treatment with biguanide compounds alone, and as biguanide differs from the sulfonylurea group both chemically and in its mode of action, it will not be discussed here

Submitted for publication July 20 1964

Case reports

Case 1 Male born in 1899 In 1950 he had an attack of cholecystitis Since 1960 he has suffered from hypertension and periodic decompensation of the heart Since 1960 he has also had a mild diabetes *treated with diet* and chlorpropamide 500 mg a day *Digitalis* chlorothiazide and potassium chloride were also given He was admitted to Borås hospital on July 1st 1963 with congestive heart failure moderate oedema of the lower limbs and enlargement of the liver The pertinent laboratory data were as follows Serum bilirubin 1.2 mg/100 ml serum alkaline phosphatase 16.5 (Bodansky units) thymol turbidity 0.1 and SGOT 40 units Electro-

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normal again The urine contained 15—20 g of glucose a day However the patient developed anuria and died on the seventh day after admission

Post mortem examination revealed a benign hypertrophy of the prostate and a secondary hydronephrosis with signs of both acute and chronic pyelonephritis The liver was enlarged showing macroscopical signs of fatty degeneration No microscopical examination of the liver was performed The hypophysis was not examined the adrenal glands showed a nodular hyperplasia of the cortex

Comments

The kidney insufficiency previously not diagnosed, was probably an important contributive cause of the hypoglycemic coma as was also a fatty degeneration of the liver The administration of glucagon 1 mg intramuscularly resulted in a rise of the blood sugar level Thus there were no signs of exhaustion of the glycogen stores of the liver A hyperplasia of the adrenal cortex was noted There were no clinical signs of Cushing s disease however and the importance of this find is uncertain

Case 3 Female born in 1887 Diabetes since 1956 at first treated with insulin but since August 1962 with tolbutamide 1 500mg a day and biguanide 50 mg a day instead Blood sugar level was 150–200 mg/100 ml and there was only slight glycosuria The patient was admitted to Borås hospital on September [illegible] 1963 with a history of cardiac decompensation in the last month and with an acute hemiparesis On admission a mild right-side hemiparesis dyspnoea and enlargement of the liver were found The pertinent laboratory data were as follows Serum bilirubin 1.5 0.8 mg/100 ml thymol turbidity test serum alkaline phosphatase SGOT and SGPT normal Bromsulphalein retention was 6

A dehydrating treatment was instituted and the patient lost 5 kg in weight in two weeks Digitalis chlorothiazide, potassium chloride and meralluridsodium were given The treatment of the diabetes was as before The patient however did not feel better, she was unwell and had a poor appetite The fasting blood sugar level varied between 90—150 mg/100 ml, then it suddenly decreased to 44 mg/100 ml The patient became diaphoretic comatous and developed paralysis of lateral gaze to the right After 15 ml 60 % glucose in water had been given intravenously, she regained consciousness During the rest of the day 650 ml 10 % glucose in water were given and in 24 hours the blood sugar level became normal again There was no glycosuria During the next five days the patient had repeated attacks of somnolence and increased sweating The blood sugar was however within normal limits Both tolbutamide and biguanide were withdrawn but in Jan 1964 chlorpropamide 250 mg a day had to be given on account of rising blood sugar levels An intravenous tolbutamide response test showed a curve of diabetic type

Comments

This patient had signs of a slight insufficiency of the liver due to heart decompensation However, the most important factor contributing to the hypoglycemic coma seems here to have been an exhaustion of the glycogen stores of the liver following an insufficient carbohydrate supply caused by her bad general condition

Chemical and physical properties of the sulfonylurea compounds

The different sulfonylurea compounds that will be discussed here are all closely related however, there are certain differences in chemical composition and in physical properties

lytes were normal Serum creatinine was 1 2 mg/100 ml

Treatment with diuretics gave an unsatisfactory result and no improvement could be observed Severe precordial pain four weeks later was interpreted as a myocardial infarction and treatment with dicumarol was started Two weeks later the patient had a big hematemesis and became shocked At that time the thrombotest value was 11 He was treated with repeated blood transfusions and his blood pressure soon became normal Three days later the patient had a hypoglycemic collapse with a blood sugar value of 40/mg/100 ml (as determined by a glucose oxydase method) He was not diaphoretic and showed no focal neurological signs

Chlorpropamide was stopped and intravenous treatment with 60 % glucose in water was immediately started, but in spite of the fact that 1,600 g of glucose were given, the blood sugar level could not be raised to normal values during the next two days 1 mg glucagon given intramuscularly had no beneficial effect During the third day, when hydrocortisone was also given, the blood sugar became normal There was no glycosuria during these days A few days later, however, the patient suddenly died with the clinical picture of circulatory insufficiency

Post mortem examination revealed a concentric hypertrophy of the heart, a diffuse myocardial fibrosis a severe nephrosclerosis enlargement of the liver and the spleen due to stasis and varices in the oesophagus No microscopical examination of the liver was performed The hypophysis showed older fibrotic changes in the central parts of the adenohypophysis The pancreas showed the picture of a chronic fibrotic pancreatitis with secondary insular hyperplasia

Comments

Thus, at autopsy this patient had a marked nephrosclerosis and a liver insufficiency following heart decompensation In spite of this he had tolerated a dosage of 500 mg chlorpropamide a day for three years His general condition rapidly deteriorated following a large hematemesis His carbohydrate supply was probably unsatisfactory at that time He then showed signs of a hypoglycemia which for three days resisted therapy with large amounts of glucose but which at last could be corrected after addition of hydrocortisone

Since the disease of the kidneys and the liver, respectively, had apparently been longstanding, this could hardly explain completely the suddenly appearing hypoglycemia The unsatisfactory carbohydrate supply probably led to an exhaustion of the glycogen stores of the liver This assumption was supported by the finding of an unchanged blood sugar value after glucagon administration Another factor which in this case may have been contributory was an insufficiency of the hypophysis, however not provoking, because the fibrotic changes were of an older date

Case 2 Male, born in 1893 Hypertension for many years He was admitted to Borås hospital on May 14th, 1963 One month before diabetes was found and on May 11th carbutamide 1,000 mg a day, was prescribed On arrival the patient was in a comatose condition diaphoretic with a deep respiration but without focal neurological signs Blood sugar was 46 mg/100 ml Carbutamide was stopped He was immediately given 35 ml 60 % glucose in water intravenously and became conscious shortly afterwards Serum creatinine was very high 10 mg/100 ml, and there were electrolyte disturbances with acidosis, the carbon dioxide combining power was 15 mEq/l Serum bilirubin was 0 4 mg/100 ml A few hours later the patient again became comatose and in spite of the fact that more than 300 g of glucose were given into the inferior vena cava, more than two days elapsed before the blood sugar level became

normal again The urine contained 15—20 g of glucose a day However the patient developed anuria and died on the seventh day after admission

Post mortem examination revealed a benign hypertrophy of the prostate and a secondary hydronephrosis with signs of both acute and chronic pyelonephritis The liver was enlarged showing macroscopical signs of fatty degeneration No microscopical examination of the liver was performed The hypophysis was not examined the adrenal glands showed a nodular hyperplasia of the cortex

Comments

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A dehydrating treatment was instituted and the patient lost 5 kg in weight in two weeks Digitalis chlorothiazide potassium chloride and meralluridsodium were given The treatment of the diabetes was as before The patient however did not feel better she was unwell and had a poor appetite The fasting blood sugar level varied between 90—150 mg/100 ml, then it suddenly decreased to 44 mg/100 ml The patient became diaphoretic comatous and developed paralysis of lateral gaze to the right After 15 ml 60 % glucose in water had been given intravenously, she regained consciousness During the rest of the day 650 ml 10 % glucose in water were given and in 24 hours the blood sugar level became normal again There was no glycosuria During the next five days the patient had repeated attacks of somnolence and increased sweating The blood sugar was however within normal limits Both tolbutamide and biguanide were withdrawn but in Jan 1964 chlorpropamide 250 mg a day had to be given on account of rising blood sugar levels An intravenous tolbutamide response test showed a curve of diabetic type

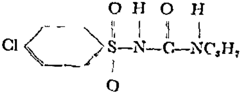
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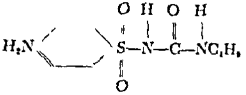
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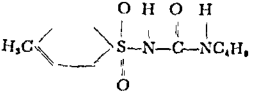
Chlorpropamide



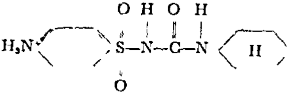
Carbutamide



Tolbutamide



Metahexamide



The most important characteristics of the different sulfonylurea compounds are summarized above and in table I.

During long term treatment with therapeutic doses of chlorpropamide and carbutamide a steady serum sulfonylurea level is reached after 3—5 days. The time for clearance of chlorpropamide and carbutamide from the blood is one to two weeks.

It is evident from table I that, basically, the properties are about the same for all these compounds, but that they differ as to the rate of disappearance.

All these compounds are considered to act in exactly the same manner. They lower the blood sugar level chiefly by promoting an increased *production* of insulin from the beta cells of the pancreas, although it seems to be certain that other factors play a part as well.

Review of the literature

The cases of hypoglycemic coma during sulfonylurea therapy reported in the literature have been divided into two groups, denoted group I and group II.

TABLE I. Important physical characteristics of the actual sulfonylurea compounds

Compound	Speed of resorption	Sulfonylurea binding protein fraction	Main mode of excretion	Half life (hrs)
Chlorpropamide	Rapid	Albumin	Urine (unchanged)	35–40
Tolbutamide	Rapid	Albumin	Urine (carboxylated)	4–5
Carbutamide	Rapid	Alphaglobulin	Urine (acetylated)	40
Metahexamide	Rapid	*Alphaglobulin*	*Urine (different metabolites)*	?

TABLE II Absolute number of hypoglycemic comas in group I and II

Compound	Group I (diabetics)	Group II (non-diabetics)
Chlorpropamide	8 (6 9 13 23 25 33 41)	5 (5 7 20 38 46)
Tolbutamide	9 (9 10 13, 17 26)	5 (13 22 39 49)
Carbutamide	7 (4 24 28 32 36 40)	1 (19)
Metahexamide	0	2 (2)
Total	24	13

Group I Patients with diabetes (4, 6, 9—11, 13 14, 17, 23—28, 32, 33, 36, 38—40)

Group II Persons either healthy (2, 5, 20, 38 46) or suffering from diseases not abnormally influencing the carbohydrate metabolism (paralysis agitans (22, 39) multiple sclerosis (13) and some psychiatric diseases (7)

These two groups have been compared as to

A Factors which may contribute to the hypoglycemic coma
  1 Frequency of hypoglycemic coma during sulfonylurea therapy
  2 Age of patients
  3 Dosages of the given sulfonylurea compounds
  4 Serum levels of the sulfonylurea compounds
  5 Frequency of complicating factors which may provoke a hypoglycemic coma

B Characteristic features of the hypoglycemic coma during sulfonylurea treatment
  1 Duration
  2 Presence of focal neurological signs
  3 Presence of excessive sweating

A FACTORS WHICH MAY CONTRIBUTE TO THE HYPOGLYCEMIC COMA

1 *Frequency of hypoglycemic coma during sulfonylurea therapy* (table II)

For obvious reasons the total number of diabetics treated with sulfonylureas is much higher than the non-diabetics The dosages are approximately the same with the exception of the cases treated with chlorpropamide (table III) Therefore the risk of falling into a hypoglycemic coma is considerably greater in patients without diabetes than with diabetes This fact might be explained by the diminished capacity of insulin production even after stimulation, among diabetics in comparison with non-diabetic patients

2 *Age of patients in groups I and II*

The mean age was higher in group I 62 years (range 37—88) than in group II, 58 years (range 22—75) Thus, hypoglycemic coma also occurred in young patients in both groups

3 *Dosage of the sulfonylurea compounds in cases with hypoglycemic coma* (table III)

There is a wide distribution of the values of the total dosages and durations

TABLE III Relations between the daily dosage the duration of therapy, the total dosage before the onset of hypoglycemic coma in groups I and II

Compound	Daily dosage (g), range and median	Duration of therapy (days), range and median	Total dosage (g) range and median
Chlorpropamide			
Group I	0 25—1 0	9—365	4 —90
	0 5	21	7 5
Group II	0 375—2 0	1—7	2—4
	2 0	2	2 6
Tolbutamide			
Group I	1 0—2 5	1—360	1—500
	1 0	87	18
Group II	0 5—1 5	2—5	1 5—4 5
	1 0	3	2 5
Carbutamide			
Group I	1 0—2 5	1—45	1 5—60
	1 5	5	10
Group II	1 5	3	4 5
	1 5	3	4 5
Metahexamide			
Group I	—	—	—
Group II	0 6	4	2 4
	0 6	4	2 4

of therapy in group I However, the coma occurs proportionately more often during the first week of treatment (one-third of the cases), particularly when carbutamide is given, the number of cases thus treated is however fairly small

In group II the range of the values of the duration of therapy and the total dosage is much less than in group I In all cases the hypoglycemia occurred during the first week of treatment

These findings seem very well to confirm the assumption that the hypoglycemic coma in a diabetic is the result not only of a certain amount of a given hypoglycemic agent but also of a defective carbohydrate metabolism, while in group II, with normal carbohydrate metabolism, the dosages are of greater importance

4 *Serum level of the hypoglycemic agent during hypoglycemic coma*

Information is only available in seven cases (4, 6, 9, 13, 22, 38, 46) The serum levels (mg/100 ml) are Group I 8 5 — 10 7 — 18 — 25 Group II 16 4 — 18 4 — 20

On the assumption that the serum levels of the different sulfonylurea compounds can be compared without further correction, there is a certain tendency to a greater range in group I than in group II, which is in accordance with previous findings regarding dosages etc (table III) However, the number of cases is small

The value of the serum concentration of a sulfonylurea compound gives only an incomplete idea of the hypoglycemic effect, as this depends upon

1 The turnover rate of the active, not protein-bound, portion determined,
2 The amount of inactive metabolites (47),
3 The tolerance, varying individually, and
4 The amount of sulfonylurea binding protein fraction (21)

Moreover, it is doubtful if there is any correlation even between the amount of the active sulfonylurea fraction in serum and the hypoglycemic effect of the compound Thus neither in diabetics nor in non-diabetics with high serum concentrations of sulfonylu

reas has hypoglycemia been mentioned when signs of overdosage have been reported (subjective muscular weakness, lassitude numbness and tingling in toes and nausea) (3 7 18) This is in accordance with the findings in experiments on different animals which show that the sulfonylurea dosage per se above a certain level does not increase the hypoglycemic effect (1, 31, 37 48)

5 Frequency of complicating factors which may provoke a hypoglycemic coma during sulfonylurea treatment

The complicating factors as evidenced from the literature are recorded in table IV

There is often a complicating factor in both groups (13 of 24 cases in group I, 5 of 13 in group II)

Several of the factors in the table may ultimately be due to an altered liver function e g starvation and alcoholism (5 6 9, 17 28) An insufficient carbohydrate supply or liver glycogen store is a possible explanation in patients during a post operative or post traumatic period (9 13 17) or those in poor general condition (28) A disturbance of the liver function may also have contributed to the hypoglycemic coma in two cases with hypoalbuminemia (7 20)

In one of the cases with alcoholism without any known liver damage the hypoglycemic coma occurred the day after acute alcoholic intoxication (33) Sulfonylurea is however not necessary in this respect Thus hypoglycemic coma may occur after an alcoholic debauch even without sulfonylurea therapy The mechanism is not clear and probably complex (16 42)

The patient with a hormonal disturbance — other than diabetes — had a chronic adrenal cortical deficiency, verified at autopsy (19) No cases with insulinoma or with a deficiency of the pituitary secretion have been reported

TABLE IV The complicating factors as evidenced from the literature

	No of cases	
	Group I	Group II
Poor condition	1	
Operation	2	
Trauma	1	
Alcoholism	1	1
Starvation	3	
Hypoalbuminemia		2
Liver diseases		1
Hormonal disturbances other than diabetes		1
Renal diseases	2	
Drugs	3	
Total	13	5

In the cases with renal insufficiency (4) an impaired excretion leading to increased half life of the sulfonylurea compound is thought to be the mechanism underlying the coma (14) However the importance of this mechanism can be doubted as the sulfonylurea concentration in serum has been found to be of minor importance in provoking the hypoglycemic coma The mechanism is probably more complex and not entirely understood

The drugs which have contributed to a hypoglycemic coma during a sulfonylurea treatment are aspirin (9) and sulfonamides (sulphaphenazole) (10)

The mechanisms are not quite clear Aspirin is known to have an "insulin-like effect" (33 34) whereas sulphaphenazole has

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Group II	0 375—2 0	1—7	2 4
	2 0	2	2 6
Tolbutamide			
Group I	*1 0 2 5*	*1—300*	*1 500*
	1 0	*87*	*18*
Group II	0 5—1 5	2—5	*1 5 4 5*
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Carbutamide			
Group I	1 0—2 5	1—45	1 5—60
	1 5	5	*10*
Group II	1 5	3	4 5
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Metahexamide			
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of therapy in group I However, the coma occurs proportionately more often during the first week of treatment (one-third of the cases), particularly when carbutamide is given, the number of cases thus treated is however fairly small

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importance in two cases (20, 27) For the remainder there was no correlation between the length of the coma and the delay of treatment Three patients died during hypoglycemic coma (19, 20, 27)

2 *Presence of focal neurological signs*

There is a high frequency of focal neurological signs in hypoglycemic coma occurring during sulfonylurea therapy Ten cases have been reported in group I, three in group II There is little doubt that these frequency figures markedly differ from those obtained in diabetics treated with insulin Apparently this is due to the higher mean age in the orally treated group than in the other and therefore also higher incidence of degenerative alterations in the cerebral vessels

3 *Presence of excessive sweating*

Sweating is considered to be characteristic of hypoglycemic coma induced by short acting insulin preparation This symptom is reported in five cases three in group I and two in group II Since most of the 37 cases so far published have been given in detail in case reports, the small number of cases with sweating is probably fairly reliable Thus the sulfonylureas in this respect are more like long acting insulin preparations giving a hypoglycemic coma without signs of excessive sweating

Discussion

Thus the *study of the literature* reveals that the hypoglycemic coma most often occurs during the first week of treatment, and that the risk of a hypoglycemic coma will increase

if the patient is a non-diabetic,

if there is a complicating liver or renal disease or insufficiency of nutrition (especially the supply of carbohydrates), or

if the patient is treated with aspirin or sulphaphenazole at the same time

The dosage and the kind of sulfonylurea compound seems to be of less importance, at least concerning diabetic patients

The clinical picture varies widely, sometimes simulating an acute cerebrovascular disease because of the presence of focal neurological signs and the lack of excessive sweating In spite of adequate treatment the coma is often prolonged or relapses in a few hours

Authors cases underline several of these facts

The dosage of the sulfonylurea compounds was the usual in all cases Two of them had been thus treated for more than one year

Possible provoking factors were present in all cases Case 1 with nephrosclerosis became comatous while in a poor general condition, then having an insufficient carbohydrate supply Binding proof of defective stores of glycogen in the liver was achieved by a glucagon test Case 2 was treated only a few days before developing a hypoglycemic coma He had a serious renal insufficiency Glucagon test revealed no lack of liver glycogen Case 3 had a slight insufficiency of the liver and became comatous during a short period with anorexia and insufficient carbohydrate supply

TABLE V Absolute number of prolonged and short hypoglycemic comas respectively, during sulfonylurea treatment

Compound	Prolonged coma		
	Continuous	Intermittent	Short coma
Chlorpropamide			
Group I	1 (14)	4 (6, 11, 25, 33)	2 (6, 41)
Group II	3 (5, 20, 38)	1 (7)	1 (46)
Tolbutamide			
Group I	2 (13, 27)	1 (9)	6 (10 17, 26)
Group II	0	3 (22, 39, 49)	2 (13)
Carbutamide			
Group I	1 (40)	3 (4, 21 32)	3 (4 28 36)
Group II	*0*	*1 (19)*	0
Metahexamide			
Group I	0	0	0
Group II	2 (2)	0	0
Total	9	13	14

no hypoglycemic effect Besides, the serum level of chlorpropamide is elevated in vitro (47) and in vivo (43) after aspirin, in vivo after sulphaphenazole (10) Probable mechanisms are competition in the tubular passages (43) or in the serum albumin sites (10)

B CHARACTERISTIC FEATURES OF THE HYPOGLYCEMIC COMA DURING SULFONYLUREA TREATMENT

There are three features which will be discussed here

1 Duration of the hypoglycemic coma
2 *Presence of focal neurological signs*
3 Presence of excessive sweating

1 *Duration of the hypoglycemic coma*

The hypoglycemic coma occurs partly in the form of short attacks, easily treated with glucose given intravenously, partly in a prolonged form, characterized by protracted or repeated bouts of coma in spite of adequate treatment (table V)

The table shows that the hypoglycemic coma is often prolonged or relapsing in spite of adequate treatment, especially in patients treated with chlorpropamide, carbutamide and metahexamide, i e those compounds having the longest half lives However, it is notable that *this type of coma will also occur among* the patients treated with tolbutamide, *as this has a much shorter half life No relevant conclusions can be drawn from* these figures as to differences between *groups I and II*

The length of the coma, measured from the onset to the regaining of consciousness of the patient varies between a few hours and more than five days Even here the diabetics (group I) seem to show the greatest range In group II all comas, with one exception, last more than 24 hours The delay before onset of treatment seems to be of

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24 LENG-LEVY J DAVID-CHAUSSE J SERRES A & PACCALIN J Coma hypoglycémique au cours d un traitement par un sulfamide antidiabétique le BZ 55 J méd Bordeaux *134* 1338 1957

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The following practically important conclusions can be drawn from both these studies

1 *Before treatment*, liver and renal diseases, hormonal disturbances other than diabetes, alcoholism and poor dietary habits should be ruled out by history-taking and simple laboratory tests

2 *During treatment* special attention should be paid to the fact that any supervening complication interfering with the general condition of the patient or the carbohydrate supply can provoke a hypoglycemic coma Moreover, caution should be observed if aspirin or sulphaphenazole are prescribed

3 Furthermore, when a sulfonylurea-treated patient is found unconscious, a hypoglycemic coma should always be suspected, as the signs of the hypoglycemic coma are very variable, sometimes simulating acute cerebrovascular disease

4 Finally, a patient with a sulfonylurea-induced hypoglycemic coma should always be kept in hospital for a few days, since a prompt result of treatment does not exclude a relapse some hours later

Summary

1 Three cases of hypoglycemic coma during sulfonylurea therapy are reported Thirty-seven previously published cases are reviewed

2 The hypoglycemic coma is often provoked by pre-existing liver or renal diseases, some drugs (aspirin and sulphaphenazole) or any supervening complication interfering with the general condition or the carbohydrate supply

3 Symptoms and signs of the hypoglycemic coma during sulfonylurea therapy are very variable, sometimes simulating an acute cerebro-vascular disease because of focal neurological signs and the lack of excessive sweating In spite of adequate treatment the coma is often prolonged or relapses in a few hours

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Maffucci's Syndrome

Dyschondroplasia with Vascular Hamartomas

By

Ragnar Berlin

Maffucci's syndrome is a condition of multiple malformations which has been almost completely overlooked in the Scandinavian literature To judge from the published reports the condition is, admittedly, not often met with, but probably the syndrome becoming more commonly known and recognized will show itself not to be an extreme rarity

The syndrome belongs to the con genital malformations linked to a cer tain germinal layer In this case we are dealing with a mesodermal dysplasia affecting the growing cartilage and con sequently also the bone formation and the bone growth (dyschondroplasia) but also affecting the formation and development of the venous system and in isolated instances also the lymphatic vessels (vascular hamartomas)

Dyschondroplasia alone has been known for many years as Ollier s disease, and in tho e cases irregular shape and dwarfism of the limbs are found The condition is characterized by failing normal ossification of the cartilage at the ends of the long extremity bones As the bones grow and tend to extend in length, islets of non-ossifying cartilage remain in the metaphyses Above all, grotesque malformations are found es pecially in the phalanxes of fingers and toes due to the development of multiple enchondromas Exostoses appear in an irregular fashion and those structures can protrude in every direction in the vicinity of the epiphyseal borderlines

This dyschondroplasia of Ollier s type appears in cases of Maffucci's syn-drome simultaneously with vascular mal formations Usually they are found as extensive birth marks , sometimes as widespread phlebectasies here and there adopting the appearance of large, com pressible bluish tumours In exceptional cases ectasies and tumourous masses

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a drawing of an amputated hand having the unmistakeable features of Maffucci's syndrome

An additional case described in more detail by Hansen (1863) was also mentioned. These two cases are obviously the same as were earlier reported by Carleton et al. and placed on their list of questionable cases of the syndrome (cases nos. 21 and 22).

Those early descriptions are, however, indecisive and are not reported as a separate disease entity; thus despite these contributions Maffucci should be reckoned the first to describe the syndrome.

Since Bean's review of 1958 (4) I have been able to collect another 17 cases reported up to 1963 (table I). This brings the total to 56 published cases of Maffucci's syndrome.

For the sake of completeness it is worth mentioning that I have found references listed in the Index Medicus to 4 additional cases from Czechoslovakia and Russia. I have not had the opportunity to consult the original papers in order to confirm that they in fact deal with Maffucci's syndrome.

Of the complete material 24 were females and 32 were males. It has been stressed however (3, 4, 6) that the number of known cases is too few to establish any sex preponderance. The accumulated evidence gives no definite pointers to a familial occurrence.

It is alarming that no less than 8 cases out of 56 have succumbed to malignant diseases, especially sarcomatous changes of cartilage and vascular anomalies. Apart from this considerable risk of eventual malignancy of the lesions, Maffucci's syndrome seems to be fully compatible with a long and useful life in spite of the grotesque malformations.

As mentioned already the syndrome apparently has been paid almost no attention in Scandinavia. One isolated case published in 1929 by Hultén and Lovén seems to belong to the disease under discussion, and recently Andrén et al. (1) reported 4 cases, only one of which was convincingly a case of Maffucci's syndrome. Mainly on these grounds I have considered it justifiable to report two additional cases of Maffucci's syndrome who have been observed during the lapse of one year in the same hospital. The first case corresponds to the classical description in every respect, the other one is most closely equivalent to cases of the type "forme fruste" or perhaps more correctly Klippel-Trenaunay's syndrome.

Case reports

Case 1. A 9-year-old boy, born in 1955. No known malformations in the family, no hereditary diseases or congenital defects whatsoever, except that the father was a diabetic. A younger brother is normal in every respect. There was no consanguinity.

During her pregnancy the mother on no occasion took any medication and she did not suffer from rubella or any other virus infection during this period.

At birth the boy exhibited a large left hand with grotesquely enlarged and deformed fingers and an extra finger hanging as an appendix laterally on the little finger. A similarly deformed right foot was found with an enlarged big toe, and the other toes were more or less grown together. Extensive haemangiomas were noted over his left hand, his left axillar region, almost over his entire right leg, on the right half of his scrotum and over a large area of his left thigh. In dif

TABLE I. Survey of Maffucci's syndrome reported in the literature

Authors	No. of cases	♂	♀
Carleton et al. (6)	18+2	14	6
Bean (3)	7+3	4	6
Bean (4)	8+1	5	4
Cameron (7)	2	1	1
Berenbaum & Tzamouranis (5)	1	1	—
Marberg et al. (12)	1	—	1
Ashenhurst (2)	1	—	1
Degos et al. (9)	3	1	2
Johnsson & Webster (10)	1	1	—
Piquet & Medioni (13)	1	1	—
Cecchi & Baccarini (8)	1	1	—
Richardson & Diddams (14)	1	1	—
Justin Besançon et al. (11)	1	1	—
Andrén et al. (1)	4	1	3
Total	56	32	24

originating from the lymphatic system are also found together with those venous malformations.

These changes are markedly asymmetric, and the bony changes and the skeletal deformities are not necessarily situated within the same areas as are the vascular malformations.

The long bones often exhibit club like thickenings at their ends because of the cartilaginous tumours at the epiphyseal borderlines. Arms and legs are further heavily deformed by bendings and fractures. Especially during the body-growth period this asymmetric development of the skeletal and vascular changes can give rise to unbelievable deformities with cauliflower-like excrescences of hands and feet.

Although all bones can be affected by these malformations they are consistently most pronounced in the distal bones of the extremities, in costae, scapulae and also in vertebrae.

As far as has been ascertained only 56 cases of the syndrome have been reported up to 1963 in the medical literature since Maffucci himself described his case in 1881. His report was later completely forgotten, and the sporadic cases now and then reported have all been considered as almost unique apparitions. The credit for having tracked down those scattered reports is due to Carleton et al. (6) who established Maffucci's priority and suggested that the condition be linked with his name. They collected all cases from the literature which they were able to find, and published in their review 18 established cases reported earlier and added two cases of their own. They mentioned also four additional cases which they considered not convincing or to be incomplete cases of the syndrome, "formes frustes".

The next systematic survey of the literature did not appear until Bean in 1955 (3) described three cases of his own observed during a number of years in the same department, and collected seven further cases from the literature published after 1942, the year when Carleton et al. completed their investigation. Later Bean (4) published one more paper on the same subject in which he again reported a case of his own and mentioned another eight cases of Maffucci's syndrome published during recent years. In addition to these cases he found out that already before Maffucci Cruveilhier in 1835 in his "Atlas d'anatomie pathologique du corps humaine" had



Fig 7 Case 1 X ray of right hand



Fig 8 Case 1 X ray of left foot

ferent places relatively large tumour like masses of a bluish tint could be observed through the skin probably cavernous haemangiomas The blood could be easily pressed away from the tumours on superficial pressure There were also noted some subcutaneous compressible masses on both sides of his chest others being more firm on palpation Biopsy from the chest tumours showed cystic lymphangiomas and from the bluish birth mark like lesions showed haemangiomas mostly of capillary type and also dilated and cystic lymphatic vessels

X ray examination revealed multiple malformations of the skeleton with syndactylia on right foot where dig II—III and IV—V were grown together On the left hand a small dig VI was seen and moreover there was a pseudarthrosis of the radial diaphysis of the left forearm

The boy was otherwise in good health and developed normally during his early childhood Repeated plastic surgery operations



Fig 9 Case 1 X ray of right foot



Fig 1



Fig 2

Figs 1 and 2 Case 1 at the age of 2 years



Fig 5 Case 1 Right foot Note haemangioma and syndactyly



Fig 3



Fig 4

Figs 3 and 4 … 1 at the age of 5 years



Fig 6 Case 1 X ray of left hand



Fig 7 Case I X ray of right hand



Fig 8 Case I X ray of left foot

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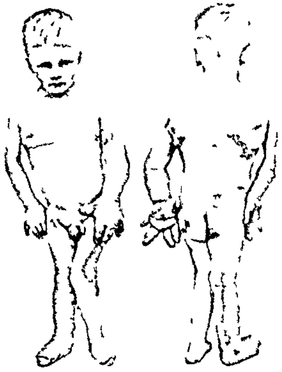


Fig 3 Fig 4

Figs 3 and 4 Case 1 at the age of 5 years



Fig 6 Case 1 X ray of left hand

grew worse X ray pictures of her femur, tibia and foot skeleton showed so called Spreissfuss and moderately coarsened bones, otherwise no pathologic changes Right-sided femoral arteriography and phlebography of her right leg revealed no special changes at that time

As the patient s condition was not recognized on that occasion an extensive examination took place including neurological and endocrinological analysis extensive X ray examination etc but it was not possible to disclose any changes compatible with a definite diagnosis

On the next admission to the hospital the possibility of a "forme fruste of Maffucci s syndrome was suggested and considered most likely

There was also noted an asymmetry of the phalanxes and the small bones of her hands and feet the right sided bones being moderately diminished and coarsened compared with the left side Thus the metatarsale I and the phalanxes of the big toe were about 7 and 3 mm longer resp and the other tarsal bones were also measured to be some mm larger on the left side The same held true for the hand skeleton as well The pictures show the appearance of the patient.

The girl has been partly incapacitated by her swollen right leg and has not been able to hold a job during recent years At present she lives with her parents doing only some slight household work

Discussion

The first case without any doubt belongs to the type Maffucci s syndrome The typical haemangiomata the grotesque deformity of the patient s left hand and right foot and the skeletal deformities all argue for the correctness of the diagnosis Admittedly the skeletal deformity is not so marked as in the classical Maffucci cases no enchondromatous changes being found with certainty, but this could be due to the patient s relatively young age and similar changes could develop later

Divergent from the classical picture of Maffucci's syndrome is also the affection of the lymphatic apparatus in this case Here too, the malformations are fundamentally of the same kind including dilatations, cystic changes and cavernous pictures As far as I have found, only three cases have earlier been described in the literature of Maffucci's syndrome where lymphatic changes have been especially noted (2, 12) In Nadell s case repeated amputations of the deformed right leg eventually disclosed a malignant lymphangioma and the patient succumbed from pulmonary metastases

The second case reported in the present paper certainly calls for discussion In this case extensive haemangiomata were found asymmetric development of both halves of the body and varicose and cavernous ectasies of the veins of the right leg with considerable swelling of the subcutaneous tissues Furthermore, less pronounced changes were found in the skeletal development of the patient s left hand and foot These criteria speak in favour of the possibility that we are in fact dealing with a forme fruste' of Maffucci's syndrome and certainly the case belongs to this group of abnormalities The case may be fully comparable with that described by Richardson and Diddams (14) It is arguable however, whether this case could be classified as Klippel Trenaunay's syndrome closely connected with Maffucci s syndrome and characterized by cutaneous angiomata varicose veins and

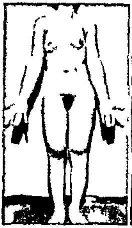


Fig 10

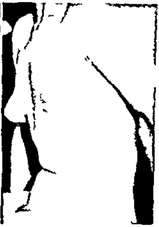


Fig 11

Figs 10 and 11 Case 2 Note the moderate hypertrophy of her right side



Fig 12 Case 2 Note widespread haemangiomas and varicosities

were performed in order to make his left hand more useful, and radiological treatment of the widespread haemangiomatous lesions was also tried for one period without any avail On one occasion he had a profuse rectal bleeding, but X ray of the colon as well as sigmoidoscopy revealed no haemangiomatous changes

At the age of seven the boy started school and he is able to follow the teaching in an intelligent and clever way He is also able to move about and to play with his classmates with only moderate difficulty with the aid of orthopaedic shoes

The adjacent pictures show the appearance of the boy at the ages of 2 years and 5 years The X ray pictures show the patient s left and right hands at the age of 3 years and his right foot Obviously these consecutive pictures say more about the general appearance of this case than a long and detailed description

In this case a chromosomal analysis was also performed and was reported entirely normal

Case 2 An 18 year old girl born in 1944 No hereditary or congenital diseases in the family Father and mother have both always enjoyed good health An elder sister suffers from asthma otherwise healthy No consanguinity was reported

She was born at full term gestation and partus being straight forward As far as can be ascertained 18 years after her mother s pregnancy there had been no untoward medication during that time and no viral infections

At birth the girl was found bluish over most parts of her left truncus, her left arm and right leg The mother noticed the discoloration on returning home after discharge from the hospital She sought medical advice but was told that the proper treatment was to wait and see The discoloration faded somewhat by degrees but was still present during her childhood and adolescence Already at birth the mother observed that the baby had unusually large hands and feet, and during her early years the girl was often ragged because of her monstrous feet At the age of 10 her right leg started to grow swollen and large varicose veins and distended blood vessels were seen Six years later she was operated on as her lesions were considered to be unusually pronounced varicosities After the operation her complaints

that in the present cases there has been no drug or other chemical influence on the growing foetus during early gestation such as attends thalidomid administration

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unilateral hypertrophy of the skeleton This syndrome has also been named by Parkes-Weber as haemangiomatous hemihypertrophy

As to the developmental factors responsible for the Maffucci changes, it seems to be clear that no signs have been ascertained pointing to the possibility of a hereditary disease In no established case has there been any familial occurrence of the syndrome (In a case reported by Steudel, 1882, a brother possibly had some suspicious-looking changes of similar kind, but he died at an early age and no conclusive evidence could be gained)

It has been much debated whether the vascular and skeletal changes of Maffucci's syndrome are in any causal connection to each other Probably this is not the case since both chief components are very often found on different parts of the body, Carleton et al as well as Bean consider them as independent occurrences but they emphasize also that it is not justifiable to leave out of account the possibility that this double mesodermal congenital defect could have a common cause in the form of some influence affecting the foetus during its early intra uterine life

Nowadays after the thalidomid disaster it seems natural in this connection to discuss also the possibility of some external factor acting on the developing foetus during the early gestation, especially the administration of drugs or other chemical compounds during the first trimester Nothing can be said about this with any certainty, but at least in the first case of the present report it can be entirely excluded that any dangerous administration of drugs took place Furthermore, no viral infections have been ascertained during the pregnancy

There are no obvious grounds for assuming that any chromosomal abnormality underlies cases of Maffucci's syndrome, and to the best of my knowledge no chromosome studies have been made earlier in these cases In our first case no chromosomal abberations were found

We may then conclude that we are dealing in those cases with a congenital dysplasia of structures of the middle germinal layer, and that the closer pathogenesis so far remains unravelled It is to be hoped that future research will elucidate these enigmatic problems and eventually add new relevant facts to our knowledge so that the development of these most incapacitating malformations can be prevented

Summary

1 A short description of the relatively rare Maffucci's syndrome is presented (dyschondroplasia and vascular hamartomas)
2 Two additional cases of this syndrome are reported, the first being entirely established and the second one probably belonging to the same group, but considered as "forme fruste" of the syndrome These cases bring the total of published cases to 56
3 Besides the classical characteristics the first case shows extensive changes of essentially the same type within the lymphatic vessels
4 Possible pathogenetic factors are discussed Especially it is emphasized

that in the present cases there has been no drug or other chemical influence on the growing foetus during early gestation such as attends thalidomid administration

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Anti-nuclear-factors (ANF) as Determined by the Immuno-fluorescent-antibody Technique

By

Vigco Faber and Preben Elling

It is an old experience that sera from patients with *lupus erythematosus disseminatus* (LED) may contain several factors having the character of antibodies, directed against substances of widely different sorts (29 36 53, 60) Thus there are antibodies against red and white blood cells and against blood platelets, and as a consequence haemolytic anaemia, leucopenia and thrombocytopenia may appear Often heterophile antibodies against different factors in the mechanism of coagulation and against gammaglobulins are found, besides non specific ' Wassermann reactions and antibodies against components of the cytoplasm from various human cells Antibodies against different bacterial substances may also exist in these sera

In 1948 Hargraves et al (35) showed that sera from patients with LED often contain a factor directed against some cell nuclear substance this could be shown by the L E cell test In the following years several reports dealt with the diagnostic value and the specificity of this test, others have shown that the L E factor is a 7 S gammaglobulin and is directed against the desoxyribonucleoprotein of the cell nucleus (e g 9, 37, 60) In Scandinavia Bastrup Madsen and Bichel (3), Bichel et al (10), Hammer et al (34) and Jokinen and Makitalo (47) made studies of some of these problems

By using other serological methods (9 29 60), it has been demonstrated that the L E cell test only proves the existence of one out of several antibodies In particular, the complement fixation technique has made a valuable contribution to the analysis of nuclear antibodies (13, 18 38, 61, 63, 64) Other methods have also been used e g the Ouchterlony technique (18, 63, 64, 69), the agglutination technique using sensitized tanned red cells or polysterene particles (14 30, 48, 59), the antigammaglobulin-consumption test (45, 57 58 60), the mixed haem absorption

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technique (23) and cutaneous anaphylaxis (19).

When Coons et al. (16) showed that proteins and among others antibodies could be conjugated with fluorochromes without loss of immunological properties, a sensitive and specific method had been devised to localize antigens in tissues. In 1954 Weller and Coons (68) introduced the "sandwich" or indirect fluorescent antibody technique. By this method it is possible to demonstrate the existence of several antibodies in serum and to localize antigens in the tissues without (as was necessary when using conventional techniques) having to isolate or fractionate these antigens beforehand.

The existence of anti-nuclear factors (ANF) in serum as determined by this method was first shown in 1957 (27, 42, 43), and Friou et al. (28) presented the first clinical material on patients with LED. Later reports (11, 24, 25, 33, 39, 41, 67), especially that of Alexander et al. (1), have extended our knowledge of the occurrence of anti-nuclear factors in serum specimens from patients with various diseases and from normal individuals. In sera from patients with LLD ANF are found in almost all cases, but these factors may also be present in many other diseases, especially those in which auto-immune mechanisms have been mentioned being of pathogenic or aetiological significance. In spite of these reports, the diagnostic and theoretical significance of these antibodies has not been fully clarified. The main purpose of the present report is to determine the occurrence of ANF in sera from normal individuals, from patients with various diseases and to discuss the diagnostic value of the test. In a later report a more detailed analysis of the different anti-nuclear factors will be presented.

Methods

The fluorescent antibody test for anti-nuclear globulin was performed by means of the indirect technique given by Weller and Coons (68). As nuclear substrate, glands from thyrotoxic patients obtained by thyroidectomy being used as a rule. The glands were cut into small pieces, immediately frozen at — 70° C. and stored at this temperature until use. The tissues were cut in a cryostate into 5μ thin pieces, mounted on slides and



a b

Fig. 1. Thyroid sections covered with serum from a normal individual (a) and from a patient with LED (b).

air-dried for 15 minutes, no fixative was used Each section was covered with a few drops of test serum and incubated in a moist chamber for 30 minutes After incubation the serum was carefully washed off in several changes of buffered saline (pH 7 2) for 20 minutes and stained with a conjugate (see below) After 30 minutes incubation, the conjugate was washed off and the slides were mounted in buffered glycerine

The anti human gammaglobulin had been prepared through repeated intra muscular injections of purified human gammaglobulin in male albino rabbits The animals were killed and each serum was subjected to immunoelectrophoresis

Rabbit sera that showed other precipitating lines than anti gammaglobulins were omitted from the final batch This final batch was precipitated twice with saturated ammonium sulphate re-suspended in 0 15 M sodium chloride and dialysed against a solution of 0 15 M sodium chloride with phosphate The concentration of proteins was estimated by means of a Biuret reaction and the protein was conjugated with fluorescein iso-thiocyanate (0 025 mg/mg protein) The conjugate was dialysed until no fluorescence could be observed in the buffer the precipitate formed during dialysing was spun down and the conjugate was passed through a column containing Sephadex G 25 The fluorescein-conjugated anti human gamma globulin was then absorbed with acetone dried guinea pig liver powder and stored at — 20 C until use

The microscope was a Leitz Laborlux with a darkground condenser a high pressure mercury vapour lamp (Osram HBO 200), a UG I exiter filter and a colourless UV absorbing barrier filter

TABLE I The incidence of anti nuclear factors (ANF) in different groups of diseases and in a control material

Diagnosis	No of pat	Per cent age pos
LFD	33	97
LED obs pro	17	53
LE discoid	16	0
Dermatomyositis	5	60
Sjogren s disease	12	42
Scleroderma	27	37
Hepatic cirrhosis	105	38
Rheumatoid arthritis	100	30
Ulcerative colitis	20	25
Hashimoto s thyroiditis	45	8
Pernicious anemia	50	6
Disseminated sclerosis	50	2
Addison s disease	7	0
Carcinoma & sarcoma	50	18
Macroglobulinaemia	12	8
Myelomatosis	30	7
Leukaemia	20	0
Sarcoidosis	20	0
Hodgkin s disease	10	0
Primary viral pneumonia	12	0
Influenza[1]	20	0
Mononucleosis	10	0
Acute myocardial infarct	150	4 5
Thyrotoxicosis	115	5 3
Donors	194	4 6

[1] Positive reaction of cold agglutinin and of influenza-complement fixation reaction respectively

Results

Altogether 1 129 serum specimens were examined The composition of the material and the results of the investigation are shown in table I In order to quantitate the content of ANF the positive sera were diluted in buffered saline The resusts are shown in fig 2

Controls

The material consists of sera from 194 blood donors all of whom had declared that they had never had any signs of auto-immune disease or any family

technique (23) and cutaneous anaphylaxis (19)

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a b

Fig 1 Thyroid sections covered with serum from a normal individual (a) and from a patient with LED (b)

patients were in an active phase of the disease On the basis of anamnestic data, the patients were classified as post-hepatic (30 patients), alcoholic (25 patients) and cryptogenic (40 patients) cirrhosis, besides biliary cirrhosis (8 pa tients) and haemachromatosis (2 patients) A positive reaction was found in 39 of the sera One half of these showed high titres and one half weak reactions No significant difference in the positive frequence in the different aetiological groups could be found

Rheumatoid arthritis

The diagnoses of the 100 patients in this group were based on the criteria suggested by The American Rheumatism Association (15) All patients were adults Seventy-nine were women The material is composed of patients with rheumatoid arthritis in the early stage as well as patients in the advanced stages of the disease Twenty-eight of the patients showed a positive ANF reaction, none of these sera showed titres over 1/100 (fig 2)

Scleroderma

The material comprised 27 patients 23 of whom showed a disseminated form, 4 a localized one The sera of the latter were all negative but 44 per cent of the former contained ANF

Morbus Sjogren

was represented by 12 cases Two of these suffered also from rheumatoid arthritis and one from scleroderma Five of the sera were ANF positive

Dermatomyositis

All the 5 patients in this group received steroids Three of the sera were ANF-positive

Lupus erythematosus discoides

None of the 16 sera from the patients in this group showed a positive reaction Two patients with a discoid form of the disease and elevated thymol reaction, elevated sedimentation rate and dubious L E cell test are not included, both sera showed weak positive reactions for ANF

Ulcerative colitis

The material comprised 20 patients with characteristic X ray, histological and proctoscopical changes One third of the patients were having heavy steroid treatment Five of the sera showed positive ANF reaction

Hashimoto's thyroiditis

The diagnoses in all 45 cases were verified histologically through biopsy or at operation All the sera but one contained complement fixing thyroid antibodies and antibodies against thyroglobulin in high titres Only 2 sera showed a positive ANF reaction

Pernicious anaemia

The material comprised 50 patients with a macrocytic anaemia, achlorhydria, a characteristic histology of the marrow and a positive Schilling test Three of these sera showed a positive reaction for ANF

Carcinoma and sarcoma

Besides sera from a few patients with rare tumour forms, sera were investi

disposition related thereto, furthermore, they had been in absolutely good health for the last three months Nine sera (4 6 %) were positive for ANF

Lupus erythematosus disseminatus

Sera from 33 patients were investigated The diagnoses were based on clinical, histological, biochemical and the usual serological criteria From table I it can be seen that 32 of the 33 sera showed a positive reaction for ANF The material comprised only patients concerning whom repeated investigations had given a reasonably certain diagnosis The L E-cell test had been positive in all patients at some stage or other of the disease, but only 2/3 of the sera were positive at the time of investigation for ANF Twelve patients were more than 50 years old, 12 patients were under 25 years of age Thirty-two of the patients were women Seventeen patients with signs of LED but with no definite criteria of the disease are placed in the group of LED obs pro Nine of the sera from these patients showed positive reaction for ANF, and 4 of these in high titres One patient with symptoms of LED after prolonged treatment with hydralazine (Apresolin) is placed in this group, high titres of ANF were shown, even several months after ceasing treatment with this drug

Hepatic cirrhosis

105 patients are included All patients showed clinical and biochemical signs of chronic damage of the liver parenchyma In 96 of the cases the diagnoses were confirmed through liver biopsy (83 cases) or at autopsy (13 cases) Sixty-five per cent of the patients were women Most of the

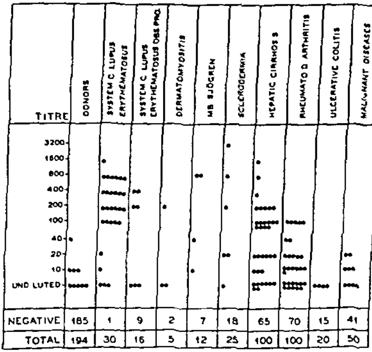


Fig 2 Titres of anti nuclear factor obtained with sera from 194 healthy donors with sera from 30 patients with hepatic cirrhosis with sera from 100 patients with rheumatoid arthritis and with sera from patients with dermatomyositis Sjogren s disease ulcerative colitis and malignant diseases

the activity of the disease, with a fall of titre during remissions It is well known that the L E factor may be non traceable during steroid treatment, and we have seen some patients in whose sera a positive ANF reaction disappeared coincidently with a clinical remission However, it is our impression that this is not a common phenomenon In the present material, 25 of the 33 patients received steroids and 8 of these 25 patients had no clinical or biochemical signs of active disease when their serum was tested Out of the 8 patients who did not receive steroids, 2 patients showed no sign of active disease one being the patient whose serum did not contain any traceable anti nuclear factor The question must remain open until more clinical data are available, especially serological investigations of patients receiving steroids with remission for long periods and patients suspected of suffering from LED

The positive frequency in the other diseases included in the present material obviously proves that the finding of ANF in a serum cannot be taken as diagnostic for one disease but shows that ANF are antibodies frequently found in the diseases called auto immune In the cases of hepatic cirrhosis in which the ANF reaction was found positive in almost 1/3 of the cases the positivity was not correlated with the type of cirrhosis as judged from anamnestic data or the histology of the biopsies It was also investigated whether signs of activity the presence of hypergammaglobulinaemia or other antibodies (rheumatoid factors etc) coincided with a positive ANF so as to be able to define or classify a special group of cirrhotic cases by serological criteria In 50 patients the ANF test was performed at monthly intervals for up to five years Positive reactions changed to negative ones in 8 patients, coincidentally with several months' remission The contrary also occurred coincidentally with a progression of the disease Twenty two patients with negative reactions when the diagnoses were made still showed the same reaction for five years independently of remission and progression and independently of steroid treatment Nine patients showed positive reactions during the whole period of observation, also independently of the course or the treatment of the disease and 2 cases varied from a positive to a negative reaction and vice versa Thus a certain degree of correlation was found between activity of the cirrhosis and the finding of anti nuclear factors in sera of some cases of cirrhosis A more detailed analysis of these relationships will be published later Corresponding investigations with regard to rheumatoid arthritis are in progress In this connection it should be mentioned that Alexander and Duthie in 1960 (1) showed that the incidence of ANF was highest among patients with signs of active disease, they claimed that the reaction might be of prognostic significance In contrast to this, Gosling et al (32) could not find any significant clinical difference between patients with rheumatoid arthritis showing positive L E cell phenomena and those who did not show this serological abnormality

It is of differential diagnostic interest that sera from some of the patient groups

gated from 4 with tumours of the breast, 4 of the stomach, 7 of the pancreatic gland, 6 of the lungs, 7 of the thyroid gland, and 9 sera from patients with reticulo sarcoma or lympho-sarcoma Nine of these 50 sera (= 18 %) showed a positive reaction for ANF

Other diseases

This material comprises 10 patients with Hodgkin's disease, 20 with lymphatic, myelocytic or stem-cell-leukaemia, 30 with myelomatosis, and 12 with macroglobulinaemia primaria (Waldenström) Furthermore 10 patients with infectious mononucleosis with a positive Paul Bunnel reaction, 20 patients with positive reaction of influenza-complement-fixation and elevated cold-agglutinin Twenty patients suffering from sarcoidosis (morbus Boeck) and 50 with disseminated sclerosis were also included Finally there were 150 patients with acute myocardial infarction and 115 patients with acute thyrotoxicosis The positive frequency of ANF in these sera is seen in table I and does not exceed that of the control material

Discussion

Up to the year 1948 patients with disseminated lupus erythematosus were mainly examined and characterized on clinical and histological grounds After the demonstration of the L F factor by Hargraves et al (35) interest switched over to serology and an extraordinary number of serological abnormalities were discovered The great variety of auto antibodies and especially the finding of antibodies directed against substances of the cell nucleus would seem to indicate fundamental disturbances of the antibody-producing system The abnormality may be correlated to the course of the disease though up to now it has not been proved that these anti bodies have any pathogenetic significance

LED has been classified as an example of a human disease with a disturbed immunological tolerance, analogous to "runt disease" in animals (21, 44) A disturbed tolerance might be due to genetic disturbances since frequent occurrence of hypergammaglobulinaemia and of anti nuclear factors in asymptomatic relatives of patients with LED has been shown (51, 52 54) A corresponding abnormality in the sera of relatives of patients with rheumatoid arthritis (50 70), with ulcerative colitis (65), and with hepatic cirrhosis (20) has been shown

The findings of the present report as to ANF in sera from LED patients correspond to those of previous reports (1, 24, 28) almost 100 per cent of these sera show positive reactions It is tempting to conclude that sera with no ANF could hardly be derived from patients with LED which would prove the diagnostic value of the ANF reaction in this disease However, objections to such a conclusion must be raised It has been previously emphasized that the material only comprises cases of LED the diagnosis of which was beyond doubt Because of the difficulty of diagnosing and defining non typical cases or early states of the disease, it is impossible to ascertain the existence of ANF in all cases of LED In addition a positive ANF finding in some cases varies quantitatively, depending on

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factors may form complexes with the 7 S globulin, as shown by McCormick and Day (55) They were able to split the macroglobulin through treatment with thioglycollate or mercaptoethanol, and after that the ANF reaction of the sera turned out to be negative Only very few of the sera from LED patients changed in the same way In the present material all ANF positive sera of rheumatoid arthritis patients showed a positive latex test thus showing the presence of macroglobulins, 2/3 of the ANF-positive sera from patients with hepatic cirrhosis showed the same serological pattern but a further analysis of the interdependency, if any, of these antibodies will appear later It is possible that a more accurate analysis of the single ANF positive serum might render the ANF reaction more specific for LED or other diseases in which the anti nuclear factors are found

The ANF reaction is a simple and reproducible serological reaction which can be performed with very small amounts of serum, thus it is well suited for screening test For the present one may conclude that in obscure cases of disease the finding of a positive ANF-reaction suggests an auto-immune disease A serum with no ANF reaction is probably not derived from a patient with LED with the reservations mentioned above Sera from patients with LED show positive reactions in almost 100 per cent of the cases

Summary

A material comprising 1,129 sera from normal individuals and from patients with various diseases has been investigated for anti nuclear factors (ANF) using the fluorescent antibody technique in order to determine the occurrence of ANF in the different groups In healthy individuals 4 6 per cent of the sera showed a positive reaction for ANF in low titres A similar or lower positivity was found in most of the groups of patients with various medical and malignant diseases

ANF was present in 97 per cent of the sera of patients with dis eminated lupus erythemato us and also frequently in rheumatoid arthritis, hepatic cirrhosis and in other "auto-immune diseases'

The diagnostic value of the ANF test is discussed

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included in the present material on account of wellknown different immunological abnormalities (dysproteinaemias etc) do not differ from those of the control group in respect of positive frequency of ANF A probable exception is the group with malignant tumours All these patients were in advanced stages of disease with metastasis, anaemia and often hypergammaglobulinaemia No coincidental collagen diseases could explain the positive reactions However, the few cases involved and the heterogenous material render it impossible even to guess whether the malignant growth in itself, the treatment with X-ray, cytostatic or psychopharmaceutical agents (e g chlorpromazine) might explain the positive reactions

In earlier reports a positive frequency of the same order found in the present material of normal individuals was found (1, 11, 33) However, Hijmans et al (39) did not find a single positive serum among 349 tested, and a few authors (8, 54)) report significantly higher positivity when using undiluted sera These authors recommend using sera diluted 1 4 or even 1 16 as a routine The differences mentioned are probably due to technical circumstances, e g questions such as inactivation of serum, presence of complement, fixation of the tissues and the 7 S or 19 S globulin content of the conjugate (40) Use of different tissues as nuclear antigens may be another explanation Recently ANF specific for skin homografts (26) and for leucocytes (22) have been demonstrated Whatever the explanation is, ANF may eventually turn out to be a constituent of normal sera demonstrable in all sera if more sensitive methods were available, this antibody may be quantitatively (and qualitatively?) elevated in pathological states (2)

The presence of ANF in the sera of diseases other than LED has been taken as a sign of a disturbed immunological tolerance in these diseases also However, it is also possible that ANF in the sera of LED patients differ qualitatively from those in other diseases The presence of ANF might possibly be due to a change of the nuclear antigen on account of mutations for example or perhaps through a hapten effect of chemicals or drugs (56)

Previously it was mentioned that the L E -cell test proves the presence of only one anti nuclear factor directed against desoxyribonucleoprotein and that several other anti-nuclear factors can be demonstrated using other serological methods The antigen/antibody reaction visualized with the fluorescent technique is probably also due to more than one factor The nuclei usually appear as a dense homogenous fluorescence, but the fluorescence may also be localized mainly to the periphery of the nucleus, or in the nucleolus Some sera give a speckled pattern, others a fine network These different morphological patterns seem to represent one circulating anti body each (4, 5, 6, 7, 12, 49, 62)

Goodman et al (31) and others (4, 46, 66) showed that anti-nuclear factors in LED sediment like 7 S globulins They are thermostable at 65° C, but thermo labile anti-nuclear factors which sediment as a 19 S may be found in diseases other than LED In the sera of patients with rheumatoid arthritis, the rheumatoid

factors may form complexes with the 7 S globulin, as shown by McCormick and Day (55) They were able to split the macroglobulin through treatment with thioglycollate or mercaptoethanol and after that the ANF reaction of the sera turned out to be negative Only very few of the sera from LED patients changed in the same way In the present material all ANF positive sera of rheumatoid arthritis patients showed a positive latex test, thus showing the presence of macroglobulins, 2/3 of the ANF-positive sera from patients with hepatic cirrhosis showed the same serological pattern, but a further analysis of the interdependency, if any of these antibodies will appear later It is possible that a more accurate analysis of the single ANF positive serum might render the ANF reaction more specific for LED or other diseases in which the anti nuclear factors are found

The ANF reaction is a simple and reproducible serological reaction which can be performed with very small amounts of serum thus it is well suited for *screening* test For the present one may conclude that in obscure cases of disease the finding of a positive ANF-reaction suggests an *auto-immune* disease A serum with no ANF reaction is probably not derived from a patient with LED with the reservations mentioned above Sera from patients with LED show positive reactions in almost 100 per cent of the cases

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Data on the Determination of SGOT and SGPT Activity in Donor Blood for the Possible Prevention of Post-transfusion Hepatitis

By

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H C Zanen and J Streefkerk

The risk of transmitting hepatitis virus to the recipient remains one of the principal problems in blood transfusion Serum hepatitis moreover is described by Allen and Sayman (1) as an affection which may endanger life particularly in patients over 40 In view of these facts it is understandable that every attempt is made to avoid this complication Pre treatment of blood to be transfused has proved not to afford adequate protection The use of one or several of the numerous liver function tests can identify some donors with disturbed liver function possibly where there is a virus infection According to current views, however this procedure does not identify those who with or without a history of hepatitis, can be virus carriers despite undisturbed liver function

Application of enzyme studies in the field of hepatology has supplied a number of determinations of greater specificity for liver tissue than those used so far Of these determinations, that of the SGOT and that of the SGPT activity have proved useful as bed-side methods

In 1959, the New York publication of Bang et al (2) appeared, which excited general interest The authors applied the SGOT determination to donors A statistical study showed that transfusion of blood with an increased SGOT activity was followed by a significantly higher incidence of hepatitis in the recipients than was transfusion of blood with normal SGOT activity According to the authors this proved the value of SGOT determination in donors as a screening method Upon reconsideration of this publication, a number of objections arise viz

1 The SGOT determination was carried out in blood kept for varying periods at room temperature before being centrifuged In blood kept in

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TABLE I Data concerning the 5 recipients with post transfusion hepatitis

Recipient	No of bottles	Tests in diagnosis post transfusion hepatitis		
		SGOT (U)	SGPT (U)	Total bilirubin (mg %)
A	1	591	1 030	20 4
B	13	280	476	5 6
C	8	372	630	6 8
D	2	250	550	9 5
E	1	640	1 110	3 2

months after the initial date and by the fact that a number of determinations had to be eliminated due to inevitable technical errors, e g a tube breaking in the centrifuge)

Only five recipients developed hepatitis From the Arnhem data, therefore the incidence of post transfusion hepatitis can with statistical treatment, be expressed in two different ways, viz

a In terms of the number of bottles administered as 1 3 per 1,000 This takes into account the survival of the recipients as seen against the background of the incubation periods of both forms of hepatitis

b In terms of the number of recipients, as 2 6 per 1 000

TABLE II *Summary of overall results 4 134 bottles tested for TA. 1 921 recipients*

Post transfusion hepatitis (n=25)				
A	1		SGOT	351 U
			SGPT	130 U
B	13	1	SGOT	48 U
			SGPT	30 U
		12	Normal	TA
C	8		Normal	TA
D	2		Normal	TA
E	1		Normal	TA
No ill-effects (n=1 916)				
3 994			Normal	TA
Total normal TA			4 005	
Increased TA			115	
Not examined			483	

TA = Transaminase activities

B Possible protection of the recipient by determination of the donor s transaminase activity

Of the 1 921 recipients five developed verified hepatitis 57 to 163 days after the date of transfusion (table I) These cases involved 25 bottles of blood (= 25 donors) 23 of these 25 donors had had normal transaminase activities One donor had a slightly increased SGOT activity (48 U) but normal SGPT activity, a second donor had an SGOT activity of 351 U and a SGPT activity of 130 U

Had we *disqualified* bottles of blood on the basis of increased transaminase activity, therefore, we might have protected a single patient who developed hepatitis after receiving only one bottle

this way, Crowley (3) maintains, SGOT-rich erythrocytes release this enzyme into the serum.

2. Of the donors described by Bang, many donated blood not primarily for idealistic reasons but for financial considerations. Among these people we may find alcoholics and drug addicts. After abuse of alcohol, the enzyme activity in the blood increases; inexpert administration of narcotics can result in hepatitis, as demonstrated by Potter et al. (4). Apart from this, it is questionable whether one can rely on the hepatitis history in the case of professional donors.

In our opinion there was good reason to establish whether, in a Dutch blood transfusion service which consists of non-professional donors, it is meaningful to screen donors by means of a correctly carried out transaminase determination.

The principal question in this context is: can recipients be protected from virus hepatitis by determination of transaminase activity in donor blood?

A second, equally important question derived from the above is: if there is increased transaminase activity in donor blood, does it spell danger to the recipient, and does a normal activity exclude this danger?

Methods

1. Determination of transaminase activity in donor blood; besides the SGOT activity, we also determined the SGPT activity which is more liver-specific.
2. Follow-up of the recipients of this blood.

The donors — members of the Arnhem Red Cross Blood Transfusion Service — are examined no more than six months before each donation according to the current Netherlands rules (i.e. history, physical examination and a simple blood count and urinalysis). In addition to this routine procedure, each tube of blood obtained during donation was immediately centrifuged, after which the serum was stored at -6 to $-7°$ C for a maximum of three days until transaminase activity was determined.

The SGOT and the SGPT activity were determined according to Reitman and Frankel (5). The above mentioned manner and duration of storage proved not to influence the enzyme activity.

Transfusions were given entirely independently of this transaminase study, i.e. without knowledge of the transaminase values determined.

The haematological work was done in three closely cooperating hospital laboratories where a SGOT activity of up to 40 U and a SGPT activity of up to 35 U were accepted as normal values.

In the follow-up study, with the indispensable collaboration of the family doctors, we were informed about the fortunes of *all* recipients within six months of the date of transfusion. This interval takes into account the incubation period of infectious hepatitis (10—60 days) and that of serum hepatitis (60—160 days).

Results

A. Incidence of hepatitis after blood transfusion

From 1st January 1961 to 1st June 1962, 2,799 donors provided a total of 4,617 bottles of blood (450 ml per bottle) for a total of 1,921 recipients. Transaminase activity was determined in 4,134 of the 4,617 bottles (the difference is explained by the fact that two of the three laboratories started haematological work 3

hol abuse was found, but only after the deliberate history taking had been completed. One of these donors was included in table I, he donated blood (with an SGOT activity of 351 U and an SGPT activity of 130 U) to recipient E who subsequently developed hepatitis. So far, questions relating to alcohol consumption have not been routine in the Arnhem service, there is consequently no factual information on the alcohol consumption throughout the donor group of this service.

Conclusion and summary

On the basis of our study we have concluded that the capacity of routine determinations of transaminase activity to prevent post transfusion hepatitis is low in the Netherlands (apart from epidemics of hepatitis) that this method must be considered practically meaningless and economically unjustified in a transfusion service. This conclusion is the opposite of that formed by Bang et al. on the basis of their USA study.

We would refer here to a Swiss publication by Stampfli et al. (6) which appeared at the time of our study. These investigators determined the SGPT activity of donors and studied the *fortunes* of the recipients of blood with increased SGPT activity. Unfortunately few of these recipients could be traced. A number of them developed fever, dyspeptic symptoms and dark urine (but no jaundice) without a definite diagnosis within a maximum of 6 weeks after transfusion. The counterpart of this study (i.e. the follow up of the large group of recipients of blood with normal SGPT activity) is lacking. Partly in view of this lack the authors' conclusion that donors with increased transaminase activity must be eliminated, in view of an increased risk of transmitting hepatitis, is untenable.

We believe that the procedure so far used in the Netherlands, with a brief history taken prior to each donation, with a physical examination and a simple blood count and urinalysis, constitutes a reliable procedure for the selection and screening of non professional donors.

To this we would add that donors who abuse alcohol should be regarded with suspicion. It is possible that a liver damaged by alcohol is more susceptible to the hepatitis virus.

Acknowledgement

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of blood (with an SGOT activity of 351 U. and an SGPT activity of 130 U.). Had we disqualified a bottle of blood with an SGOT activity of 48 U. (and normal SGPT activity), we might have protected a second patient, who developed hepatitis after receiving a total of 13 bottles of blood. This possible protection could have been achieved at the cost of 4,134 SGOT and as many SGPT determinations.

THE OVERALL TRANSAMINASE STUDY

A total of 4,134 bottles were examined; 117 bottles contained blood with increased transaminase activity (table II). Two bottles were previously mentioned under B. The remaining 115 bottles derived from 109 donors, including 5 who more than once donated blood with increased enzyme activity. Transfusions of these 115 bottles with increased transaminase activity, however, had no untoward effect on the recipients.

Had we disqualified on the basis of increased transaminase activity, then these 115 bottles would have had to be thrown away.

After transfusion of 4,005 bottles with normal transaminase activity, three of the recipients nevertheless developed hepatitis.

JAUNDICE IN THE HISTORY OF DONORS

Jaundice is mentioned in the history of 149 of the 2,799 donors. It is difficult to establish whether the cause of the jaundice was always hepatitis. In all cases the illness occurred more than five years ago. The majority of these donors had been jaundiced during the period 1940—1945, when the hepatitis incidence was very high. Correlating the 149 donors with the fortunes of the corresponding recipients, we find the following:

a. Two donors were included in the series of 13 and 8 donors respectively (table I), whose blood was transfused to two patients (B and C) who later developed hepatitis. At the time of donation these two donors had a normal transaminase activity. One donor, in whose family jaundice prevailed at the time of donation, was also included in the above mentioned series of 13 donors. The donor herself had a normal transaminase activity at the time of donation. It must be pointed out that the fact that this donor donated her blood at that time was a result of an oversight in the blood transfusion service.

b. Eight donors belonged to the group of 109 whose blood showed increased transaminase activity at the time of donation. No hepatitis developed in the recipients after transfusion.

c. The remaining 139 donors with a history of jaundice showed a normal transaminase activity at the time of donation. The recipients manifested no hepatitis.

It can be pointed out that the incidence of hepatitis in the area from which the donors were recruited was low at the time of this study, the risk that the donors were donating during the incubation period of hepatitis was consequently small.

ALCOHOL CONSUMPTION BY THE DONORS

In four donors with increased transaminase activity, evidence of severe alco-

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Calcium and Phosphorus Metabolism in Sarcoidosis

By

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The first to report their observations on calcium and phosphorus metabolism in sarcoidosis were Harrel and Fisher (14), in 1939 All of their ten patients had normal serum phosphorus but six had increased serum calcium values Interest in serum calcium has been maintained The incidence figures reported for hypercalcemia in sarcoidosis have exceeded 20 per cent apart from a few exceptional series These latter reports have raised doubts as to whether hypercalcemia is in fact so common as is generally stated (10 20 28) Serum phosphorus, on the other hand, has received very little attention Besides Harrel and Fisher (14), only Longcope and Freiman (26) have published a study based on a reasonably large number of cases

Available data (1 3 4 16 19 28) on urinary calcium and phosphorus are based on only a few balance studies The case materials for these studies were selected, consisting altogether of less than 20 cases Nevertheless the opinion emerging from the literature is that hypercalciuria is very frequent in sarcoidosis The Washington conference on sarcoidosis in 1960, describing the disease, placed hypercalciuria among important laboratory findings immediately after the Kveim reaction and anergy to tuberculin, and before increased serum globulins (17)

Since the statements in the literature are in part controversial and incomplete, it seems justified to present our observations in regard to serum and urinary calcium and phosphorus and the closely associated serum alkaline phosphatase In addition, some observations on serum and urinary magnesium in sarcoidosis will be reported

Material and methods

The material consisted of 60 patients with sarcoidosis treated in the Department of Dermatology University Central Hospital Helsinki in the period 1955—1964 Eighteen of the patients were male and 42 female They varied in age from 9 to 67 years with an average of 33 5 years Diagnosis was con

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the first determination ranged from 8 6 to 13 0 mg% in the sarcoidosis group and 9 0 to 11 1 mg% in the control group The means were of the same order in the first determinations in the sarcoidosis group 9 95 mg% with SD of 0 61, in all 207 determinations 9 91 mg% with SD of 0 56, and in the control group 9 81 mg% with SD of 0 41

In only two sarcoidosis patients did the serum calcium exceed 11 0 mg% (11 4 and 13 0 mg%) at the first determination but in repeated tests after two months the value was normal in both of these cases (9 6 and 10 0 mg%) In the control group one case showed a value over 11 mg% (11 1 mg%)

A total of 209 determinations of serum phosphorus were made in 57 sarcoid patients and one determination in each of 75 controls The mean serum phosphorus value for the latter was 3 77 mg% with SD of 0 51 and for the sarcoid cases 3 46 mg% (first determination) with SD of 0 51 Thus the difference between the means was 0 31 mg % which using the t test, was statistically significant ($p < 0\ 01$) The mean of all values in the sarcoidosis group was 3 52 mg% with SD of 0 53

Fig 2 shows the frequency distribution of the phosphorus values In the sarcoidosis group the frequency distribution clearly shifted towards low values as compared with the controls Fig 3 gives separately the frequency distribution in chronic (over 2 years) and subacute sarcoid cases On comparing these it is clearly apparent that the chronic cases yielded almost exclusively low values According to the first determination there were 8 sarcoid patients (14 %)

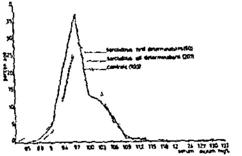


Fig 1 Frequency distribution of serum calcium in 60 sarcoid patients and 100 controls

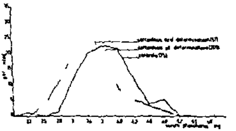


Fig 2 Frequency distribution of serum phosphorus in 57 sarcoid patients and 75 controls

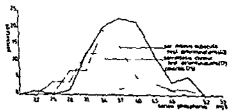


Fig 3 Frequency distribution of serum phosphorus in 17 chronic and 40 subacute sarcoid cases and 75 controls First determinations

and two controls (3 %) with values below 3 mg% Permanently depressed serum phosphorus was encountered in two sarcoid patients both chronic cases

The serum magnesium was determined in 45 sarcoid patients, the number of tests totalling 90 The mean of the first determinations was 1 91 mg% with SD of 0 35 and of all determinations

TABLE I *Manifestations of sarcoidosis in 60 patients and the results of biopsy, Kveim test and Mantoux*

Manifestations	No	Percentage
Skin	31/60	52
Erythema nodosum	16/60	27
Lung incl hilar adenopathy	54/60	90
hilar adenopathy only	23/60	38
Peripheral lymph nodes incl prescalenic nodes	21/60	35
Bone	5/60	8
Eye	8/53	15
Mucosal (biopsy positive)	5/46	11
Other	9/60	15
Biopsy evidence	45/60	75
Kveim positive	54/60	90
Mantoux		
Neg to 0 1 TU of OT	58/58	100
Neg to 1 TU of OT	53/58	91
Neg to 10 TU of OT	29/58	50
Neg to 100 TU of OT	18/58	31

firmed in all cases by a positive Kveim test and/or biopsy None of the patients had received calciferol for the last six years or steroid therapy for the last three months before the laboratory studies were made The manifestations of the disease are given in table I It also shows the results of the Kveim test and biopsy as well as the response of the patients to the Mantoux test

One hundred persons served as controls, 44 healthy volunteers and 56 patients under treatment at the Dermatological Department for various diseases without internal medication

Serum calcium was determined up to 1960 with the $KMnO_4$ titration method (22) and later with the complexometric EDTA titration method (36) The results with these two methods were considered comparable since the values obtained with each from one and the same serum sample in 57 control cases were on an average the same The urinary calcium was measured by the complexometric EGTA titration method (34) Phosphorus determinations were made on both serum and urine according to a modified Fiske-Subbarow method (11) The serum and urinary magnesium was estimated according to Orange and Rhein (32) Serum alkaline phosphatase was determined up to 1960 by the method of Bodansky (6) and later by that of Bessey and Lowry (5)

The blood samples were taken at 8—9 a m after an overnight fast, the 24-hour urine was collected between 8 o'clock in the morning and 8 a m the next day The ratio of males to females was the same in the sarcoidosis and the control group In this way, as far as possible the physiological variations were eliminated that occur in the concentrations of serum phosphorus and in the urinary calcium and phosphorus in the two sexes and at different times of day For the urine studies the patients were kept on a diet with a calcium content of 800—900 mg/24 hours (i e normal intake according to Shohl (38)), and the diet was started one day before the collections were begun

The following normal values were used as reference points in evaluating the results serum calcium 9 0—11 0 mg%, phosphorus 3 0—4 3 mg%, magnesium 1 6—2 5 mg%, alkaline phosphatase 0 8—2 9 B—L units and 2 0—4 5 Bodansky units The upper limit for urinary calcium was 300 mg/24 hours, for phosphorus 1,300 mg/24 hours and magnesium 200 mg/24 hours

Results

Altogether 207 determinations of serum calcium were made in 60 sarcoidosis patients and one determination in each of 100 control persons The results are shown in fig 1 as frequency polygons Their peaks coincide and the frequency distribution is similar, approximating to the normal distribution The limits in

TABLE II Hypercalcemia in sarcoidosis in the United States Small case materials published before 1950 are omitted

Authors		Total No of cases	Hypercalcemia No	Hypercalcemia %	Ca mg % exceeding
1 Longcope & Freiman	(26)	23	6	27	11 0
		21	5	24	12 0
2 Cummings et al	(8)	113	40	35	11 0
3 Israel et al	(18)	160	45	28	11 4
4 Labow et al	(23)	100	21	21	11 0
5 Mayock et al	(29)	97	18	19	11 0
6 Taylor et al	(41)	345	57	16 5	10 9

Discussion

Studies in the U S A have shown the incidence of hypercalcemia in sarcoidosis to be 17—35 % (table II) In England, however, hypercalcemia seems to be rare a transient increase in blood cal cium was found in only one of Ferguson s and Paris 29 cases of sarcoidosis (10) and a persistently raised calcium level only once in James s series of 150 pa tients (20) Mather (28) also of Eng land reported on 86 sarcoidosis cases, including four exceeding his normal range of 8 5—11 mg% (highest value 12 3 mg%) but also four with subnormal values (lowest 7 8 mg%)

Our 60 untreated cases of sarcoidosis collected from Finland comprised only two with a transient elevation of serum calcium (11 4 and 13 0 mg%) in the first test but also one of the controls had the value 11 1 mg% The frequency distribution of the calcium values showed no difference between the sarcoidosis and the control group as seen in fig 1 Thus our results like the English ones dif fered from those obtained in the U S A

The explanation that first suggests it self is the predominance of negroes in American studies Indeed, in the study of Labow et al (23) the incidence of hypercalcemia in 73 negroes was 25 % as against only 11 % for 27 whites The results of Taylor et al (41), on the other hand showed an entirely opposite picture hypercalcemia occurred in 15 % of 283 negroes and in 23 % of 62 whites So the influence of race seems open to question

Naturally the incidence of hyper calcemia rises if account is taken of only the highest value of several calcium determinations for one and the same pa tient This is what some investigators have done, Leitner (25) has stated that blood calcium can be increased in sar coidosis at *certain* phases only, thus determinations *performed* only once may often give rise to errors in judg ment

Temporarily increased calcium may also be explained by laboratory errors This already appeared definitely in Ehr

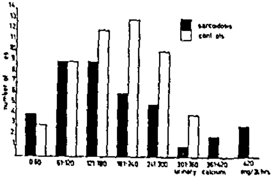


Fig 4 Urinary calcium in 37 sarcoid patients and 51 controls

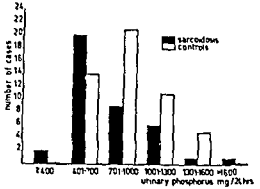


Fig 5 Urinary phosphorus in 37 sarcoid patients and 51 controls

2 00 mg% with SD of 0 32 The first test yielded depressed values in 4 patients and elevated in one There were no permanently high or low values

The serum alkaline phosphatase was studied in 51 sarcoid patients A value exceeding the normal was found in 5 patients and a subnormal value in 2 The maximum recorded was 4 5 B—L units and the minimum 1 4 Bo units Increased values were thus found in 10 % and decreased values in 4 % This result does not permit the conclusion that alkaline phosphatase typically increases in sarcoidosis

Determinations of urinary calcium and phosphorus were done in 39 patients with sarcoidosis and 51 controls The results for calcium appear in fig 4 The urinary excretion of calcium in sarcoid cases was in the main slightly lower than in the controls However, values exceeding 300 mg/24 hours were encountered in 6 sarcoid patients, in three of whom they were over 420 mg/24 hours These last three values were immediately checked on 2—3 repeated urine collections and the values were of the same order of magnitude In 4 control cases calcium excretion also exceeded 300 mg/24 hours, the highest value, however, only amounted to 324 mg/24 hours Consequently the mean calcium excretion did not differ appreciably in sarcoidosis it was 189 mg/24 hours and in the controls 187 mg/24 hours The incidence of hypercalciuria, however, was 15 % in sarcoidosis against 6 % in control cases There was no difference in the values between subacute and chronic cases of sarcoidosis

The results for urinary phosphorus are shown in fig 5 The frequency distributions are not uniform low values were more frequent in the sarcoidosis group than in the controls The difference is distinctly seen also from the means sarcoid group, males 1,013 mg/24 hours, females 639 mg/24 hours, altogether 764 mg/24 hours, in the control group these figures were, respectively 1,069 mg/24 hours, 786 mg/24 hours, and 889 mg/24 hours However, the difference was not statistically significant Phosphorus clearance was normal in both the groups

Urinary magnesium was determined in 17 sarcoid patients None of the values obtained exceeded *200* mg/24 hours

in North Carolina, U S A , where the patients of Taylor et al lived

The calcium metabolism is also influenced by renal function and serum albumin concentration To exclude insufficiency of the kidneys, serum creatinine or serum non protein nitrogen was determined in 45 sarcoidosis patients All these values were normal The controls at the Dermatological Department had been so selected as to include none with systemic diseases or kidney disorders Determinations of serum creatinine were carried out in 28 control cases with normal results The concentration of albumin in serum was normal in all 60 cases of sarcoidosis (3 2—5 8 g/100 ml)

The serum phosphorus content in sarcoidosis has stimulated much less interest than has serum calcium A number of cases have been reported with normal serum phosphorus in spite of hypercalcemia (7 14 30, 37) yet Longcope and Freiman s study (26) was the first to include serum phosphorus determinations on as many as 40 sarcoidosis patients These writers referred to their results briefly as follows Some variations and occasional low values may occur but these are unusual and phosphorus values ranged from 2 4 to 4 8 mg 100 cc

We have found only a few studies containing determinations of urinary phosphorus excretion in sarcoidosis and even these were performed in connection with the calcium balance studies referred to above

In our series of sarcoidosis in which serum calcium was normal there was distinct difference in serum phosphorus values between the sarcoidosis group and the control group in regard to frequency distribution (fig 2) The sarcoid group comprised so many low values compared with the control group that the difference between the means, 0 31mg%, was statistically significant Depressed values were found chiefly in patients with chronic sarcoidosis, as shown in fig 3 There was no difference in serum phosphorus values between healthy volunteers and control patients

The 24 hour phosphorus excretion, in the 39 sarcoid cases thus tested, was on an average somewhat lower than in the controls

A comparison of the serum and urinary calcium, phosphorus and magnesium and serum alkaline phosphatase in the patients with sarcoidosis seemed to indicate that these values lack any interdependence This finding rules out a number of conditions with pathological calcium and phosphorus metabolism It is possible that the low phosphorus values bear some relationship to the abundant synthesis of phospholipids in sarcoidal granulomas

Summary

Calcium and phosphorus metabolism was studied in 60 untreated sarcoidosis patients Serum calcium was within normal limits and the frequency distribution of the patients' calcium values did not differ from that of 100 controls On the other hand the number of low phosphorus values recorded in sarcoid cases was so much greater than in the controls that the difference of the means was statistically significant Depressed values

ström's (9) studies from 1934 Belk and Sunderman (2) sent the same calcium solutions (6 6 and 12 6 mg%) for examination to 44 clinical laboratories in Pennsylvania One of four laboratories made "gross errors" (≥ 2 5 mg%), and satisfactory results (≤ 0 5 mg%) were only obtained from one third of them Israel (18) stated in fact 'It is probable that technical variations account for many of the transient elevations reported in serum calcium concentrations" Of his 160 patients with sarcoidosis, 28 % had *transient high calcium values (over 11 6 mg %)*, but so had 19 % of the 'non sarcoidal" *controls Only 4 patients* with sarcoidosis had persistently raised values

Even though hypercalciuria was accorded importance in the definition of sarcoidosis at the Washington Conference (17), we have not found any reports illustrating its incidence in this disease Those 39 sarcoid patients for whom we determined the calcium content of the 24-hour urine included six with a value exceeding 300 mg/24 hours (three patients over 420 mg/24 hours), the maximum value *being 591 mg/24 hours* Four control patients also had hypercalciuria but the highest value was 324 mg/24 hours Of the sarcoid cases with a hypercalciuria of more than 420 mg/24 hours, one showed PBI of 10 9 μg%, and hyperthyroidism may well have been responsible for the hypercalciuria There was no correlation between the calcium values in urine and in serum

Abnormalities in calcium metabolism in sarcoidosis *have been accounted for* by the statement that sarcoid patients, or *at least some of them*, are excessively sensitive to vitamin D This idea already came to the fore when calciferol was being tried for treatment of this condition (12, 13, 24, 27, 35, 40), and it seems to be supported by calcium balance studies made on a number of patients (1, 3, 4, 16, 19, 28) It seems probable that sensitivity to calciferol is increased in some sarcoidosis patients, yet it should be kept in mind that a great number of patients with lupus vulgaris showed intoxications in connection with vitamin D treatment (21, 33, 39) and that intoxication developed in *only one of Nelson's* eight calciferol treated cases of sar*coidosis* (31) Of the seven sarcoid cases treated with calciferol in our hospital (dose 1,500,000 IU/week), three had transient renal insufficiency but in one who received a total of 42 5 million IU of calciferol during 1 1/2 years this large dose failed to produce even hyper calcemia The basic cause of calciferol hypersensitivity has not been detected

In an attempt to explain hypercalcemia in sarcoidosis it has been assumed that sarcoidal tissue might produce *some* calciferol like substance in large amounts *(15), but there is no evidence in favor of* this It has also been stated that the increased vitamin D supply to the organism by ultraviolet rays could suffice to raise the blood calcium level in sarcoidosis (28) The serum calcium in the sarcoid patients of Taylor et al (41) was on an average 0 37 mg% higher in the summer (June—Aug) than in the winter (Dec —Feb) In our study the *calcium* values were highest in late autumn (*Nov —Dec*) and lowest in summer (June—Sept) However, there is considerably less sunshine in Finland than

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occurred mainly in cases of chronic sarcoidosis In 39 patients studied, the 24-hour urinary excretion of phosphorus was also on the average somewhat lower than in the control persons though there was no statistically significant difference Urinary calcium excretion exceeded 300 mg/24 hours in 6 of these 39 sarcoid patients but also in 4 of 51 controls The serum magnesium was determined in 45 and urinary magnesium in 17 patients with sarcoidosis No pathological values were found Determinations of serum alkaline phosphatase were made in 51 sarcoid patients A raised value was obtained in 5 (maximum 4 5 B—L units) and a depressed value in 2 (minimum 1 4 Bo units) No correlations were demonstrable for the above laboratory findings It is possible that the low serum phosphorus stands in some relation to the abundant synthesis of phospholipids in sarcoidosis

Acknowledgements

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Acta Medica Scandinavica Vol 177, fasc 3, 1965

From the Laakso Hospital (Head K. Kivikanervo M.D.) and the Second Medical Department (Head Ilmari Vartiainen M.D.) University of Helsinki, Helsinki, Finland

Malabsorption and Deficiency of Vitamin B_{12} Caused by Treatment with Para-aminosalicylic Acid

By

O. Heinivaara and I. P. Palva

In a preliminary report published in this journal (1) the present writers demonstrated a decreased absorption of vitamin B_{12} measured by the Schilling test in patients receiving treatment with para-aminosalicylic acid. The present paper reports the results of Schilling tests in a larger series of patients and the serum vitamin B_{12} concentrations in patients receiving long term PAS treatment.

Material and methods

Twenty-one patients receiving either pure para-aminosalicylic acid or one of its derivatives (12–16 g daily) for pulmonary tuberculosis were subjected during this treatment to the Schilling test. Schilling test II with intrinsic factor (IF) was also performed on the majority of these patients. Additionally the ordinary Schilling test was made on all the patients either before the commencement of the PAS treatment or 2—6 weeks after its discontinuation. Most of the patients received other antituberculous drugs concurrently but invariably the only medicine added or withdrawn during the test period was PAS.

The Schilling test was performed according to Schilling (4) using 0.7 μg of vitamin B_{12} ^{60}Co (Abbott Lab., North Chicago, Ill.). In the Schilling II test one capsule of purified IF preparation (Abbott Lab.) was used as the additive.

The serum vitamin B_{12} concentration was determined in 74 patients on long term PAS treatment. The Schilling test had not been performed on these patients and, as far as was known, they had not in other connections received vitamin B_{12} in any form. The determination was also made in a control group consisting of 44 healthy persons who had not received PAS treatment. The B_{12} assay was performed by the method of Ross (3) as modified by Palva (2), using *Euglena gracilis* strain Z as test organism.

Results

Effect of PAS on the Schilling test

Table I shows the Schilling values during PAS therapy and without PAS. With the exception of two patients, the Schilling value during PAS treatment was clearly lower than when the patient did not receive PAS. The mean value during

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The Schilling test was performed according to Schilling (4) using 0 7 μg of vitamin B_{12} ^{58}Co (Abbott Lab, North Chicago Ill) In the Schilling II test, one capsule of purified IF preparation (Abbott Lab) was used as the additive

The serum vitamin B_{12} concentration was determined in 74 patients on long term PAS treatment The Schilling test had not been performed on these patients and as far as was known they had not in other connections received vitamin B_{12} in any form. The determination was also made in a control group consisting of 44 healthy persons who had not received PAS treatment The B_{12} assay was performed by the method of Ross (3) as modified by Palva (2) using *Euglena gracilis* strain Z as test organism

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TABLE I Schilling test values during PAS treatment and without PAS *Schilling I* = ordinary test, Schilling II = Schilling test with intrinsic factor

Pat no	During PAS treatment		Without PAS
	Schilling I	Schilling II	Schilling I
1	1 5	—	11 5
2	9 4	—	18 0
3	7 0	—	21 0
4	1 8	—	9 0
5	1 8	—	19 8
6	1 4	6 6	13 2
7	3 4	2 8	24 0
8	11 5	8 6	20 0
9	2 5	1 8	5 8
10	3 8	5 6	20 4
11	3 6	2 8	10 0
12	2 9	2 4	7 2
13	5 0	4 1	14 4
14	7 0	2 8	15 8
15	13 4	—	12 2
16	5 8	7 0	13 9
17	3 5	4 7	8 9
18	7.5	7 6	15 1
19	1 0	2 2	6 3
20	8 2	7 4	11 6
21	8 0	9 3	7 9
Mean	5 4	5 2	12 4

PAS therapy was 5 4 per cent and without PAS 12 4 per cent It was further observed that the addition of IF did not improve the lowered Schilling values during PAS therapy, the mean value of the Schilling tests with IF being only 5 2 per cent It should be noted that no differences were found between the PAS preparations used

The Schilling test does not measure the absorption of vitamin B_{12} directly The lowered values may indicate a change in either the absorption, storage or renal excretion of radiovitamin B_{12} If the reason for the lowered values, however, were a change in storage or in renal excretion, greater quantities of vitamin B_{12} than usual should be retained in the organism The two cases of megaloblastic anaemia developing during PAS treatment, mentioned in our preliminary report (1), and the low serum vitamin B_{12} concentrations presented below in patients receiving PAS treatment are evidence to the contrary, indicating a deficiency of vitamin B_{12} in the body during PAS therapy In our opinion the interpretation therefore appears justified that the lowered Schilling values are a result specifically of an impaired absorption of vitamin B_{12}, and that the PAS treatment probably is the factor causing in some way the reduced absorption

Although PAS is known to cause gastric distress very frequently, the B_{12} absorption disturbance can hardly be due to a possible PAS induced gastritis and reduced secretion of IF, since the addition of IF did not normalize the Schilling values Another reason that may be considered for the lowered Schilling values during PAS treatment is some direct chemical reaction between PAS and vitamin B_{12} that may inhibit the intestinal absorption of vitamin B_{12} To study this possibility an ordinary Schilling test was made on 12 subjects who had not received PAS A few days later the test was repeated, each subject being given in this connection 5 g of PAS The results presented in table II show that a single dose of PAS had no effect on the absorption of vitamin B_{12} This is in agreement with our observa-

TABLE II Results of Schilling tests made with a single dose of 5 g of para aminosalicylic acid additive

Pat. no	Ordinary Schilling test	Schilling test with 5 g PAS
31	11 4	12 7
32	18 5	15 9
33	14 7	11 8
34	11 4	6 9
35	14 2	11 1
36	10 4	8 0
37	13 6	12 5
38	11 5	19 4
39	16 2	15 5
40	14 2	13 4
41	28 0	18 7
42	14 8	9 1
Mean	14 9	12 9

TABLE III Serum vitamin B_{12} levels in controls and in patients receiving PAS treatment

Group	Serum level of vitamin B_{12} (pg/ml) 0—99	100—199	> 200
Control group no PAS treatment (n = 44)	2 (5 %)	5 (11 %)	37 (84 %)
Group with PAS treatment ≤ 1 year (n = 42)	20 (48 %)	15 (36 %)	7 (16 %)
Group with PAS treatment > 1 year (n = 32)	25 (78 %)	5 (16 %)	2 (6 %)

tion that the PAS treatment must be of two to six weeks duration before the absorption of vitamin B_{12} is disturbed and the Schilling values are lowered

Serum level of vitamin B_{12}

The serum vitamin B_{12} concentrations in control subjects and in patients receiving PAS treatment are presented in table III Clearly pathologic values (< 100 pg/ml serum) were obtained in only 5 per cent of the controls, in nearly one half (48 %) of the patients given PAS treatment during less than one year, and in as many as three fourths (78 %) of the patients under treatment for over one year The differences were statistically significant ($p < 0\ 01$) Accordingly, the serum level of vitamin B_{12} decreases during treatment with PAS and the decrease is proportional to the duration of treatment

It deserves to be noted that the group of patients receiving PAS therapy for over one year — the longest periods being nearly three years — included no case of definite megaloblastic anaemia despite the remarkably low serum vitamin B_{12} values The mean corpuscular haemoglobin of some patients was, it is true, as high as 38 and the erythrocytes exhibited anisocytosis However, the haemoglobin in every case was over 10 g/100 ml and no megaloblastosis was evident in the bone marrow According to Palva (2), even the carriers of *Dibothriocephalus latus* do not — despite their low vitamin B_{12} values — manifest megaloblastosis of the bone marrow as long as the haemoglobin level is over 10 g/100 ml

Discussion

In our opinion the following conclusions may be drawn from the results of our studies Treatment with PAS causes de-

TABLE I Schilling test values during PAS treatment and without PAS Schilling I = ordinary test, Schilling II = Schilling test with intrinsic factor

Pat no	During PAS treatment Schilling I	Schilling II	Without PAS Schilling I
1	1 5	—	11 5
2	9 4	—	18 0
3	7 0	—	21 0
4	1 8	—	9 0
5	4 8	—	19 8
6	1 4	6 6	13 2
7	3 4	2 8	24 0
8	11 5	8 6	20 0
9	2 5	4 8	5 8
10	3 8	5 6	20 4
11	3 6	2 8	10 0
12	2 9	2 4	7 2
13	5 0	1 1	14 4
14	7 0	2 8	15 8
15	13 4	—	12 2
16	5 8	7 0	13 9
17	3 5	4 7	8 9
18	7 5	7 6	15 1
19	1 0	2 2	6 3
20	8 2	7 4	11 6
21	8 0	9 3	7 9
Mean	5 4	5 2	12 4

PAS therapy was 5 4 per cent and without PAS 12 4 per cent It was further observed that the addition of IF did not improve the lowered Schilling values during PAS therapy, the mean value of the Schilling tests with IF being only 5 2 per cent It should be noted that no differences were found between the PAS preparations used

The Schilling test does not measure the absorption of vitamin B_{12} directly The lowered values may indicate a change in either the absorption, storage or renal excretion of radiovitamin B_{12} If the reason for the lowered values, however, were a change in storage or in renal excretion, greater quantities of vitamin B_{12} than usual should be retained in the organism The two cases of megalo blastic anaemia developing during PAS treatment, mentioned in our preliminary report (1), and the low serum vitamin B_{12} concentrations presented below in patients receiving PAS treatment are evidence to the contrary, indicating a deficiency of vitamin B_{12} in the body during PAS therapy In our opinion the interpretation therefore appears justified that the lowered Schilling values are a result specifically of an impaired absorp tion of vitamin B_{12}, and that the PAS treatment probably is the factor causing in some way the reduced absorption

Although PAS is known to cause gas tric distress very frequently, the B_{12} ab sorption disturbance can hardly be due to a possible PAS induced gastritis and reduced secretion of IF, since the addi tion of IF did not normalize the Schilling values Another reason that may be considered for the lowered Schilling values during PAS treatment is some direct chemical reaction between PAS and vitamin B_{12} that may inhibit the intestinal absorption of vitamin B_{12} To study this possibility an ordinary Schilling test was made on 12 subjects who had not received PAS A few days later the test was repeated, each subject being given in this connection 5 g of PAS The results presented in table II show that a single dose of PAS had no effect on the absorption of vitamin B_{12} This is in agreement with our observa

quired at least two weeks' treatment with PAS Prolonged PAS therapy was thus observed to reduce the absorption of vitamin B_{12}, as a result of which clearly reduced serum vitamin B_{12} concentrations were seen in 74 patients who had received long term PAS therapy The mechanism by which PAS may cause a disturbed vitamin B_{12} absorption and the reasons for the relatively rare appearance of the clinical manifestation of vitamin B_{12} deficiency, megaloblastic anaemia, are discussed

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creased intestinal absorption of vitamin B_{12}, as a result of which many patients develop during prolonged treatment a vitamin B_{12} *deficiency of the body, manifest* as a pathologically low serum level of vitamin B_{12}

Concerning the mechanism by which PAS leads to an impaired absorption of vitamin B_{12}, our results indicate that the phenomenon concerned cannot be a reduced secretion of IF nor such a direct chemical reaction between PAS and vitamin B_{12} as would itself inhibit the absorption of vitamin B_{12} Since the development of the absorption disturbance generally needs at least two weeks' treatment with PAS, the thought arises that PAS may disturb in some way the enzymic functions of the intestinal wall and thereby produce the absorption disturbance Further studies are needed for the clarification of this point, as well as of the question whether the absorption of other substances in addition to vitamin B_{12} is disturbed Studies now in progress and to be published later point to the probability that this is a specific vitamin B_{12} absorption disturbance

Haemolytic or aplastic anaemia, leukopenia, agranulocytosis or thrombocytopenia are stated to occur as complications of PAS therapy (5) On the other hand, the literature contains no report of megaloblastic anaemia in association with PAS treatment with the exception of the two cases described in our preliminary report (1) The rare occurrence of megaloblastic anaemia — despite the pathologically low vitamin B_{12} concentrations of the serum during PAS therapy — may be ascribed to a number of factors 1 The vitamin B_{12} stores of the human body are large Only a prolonged inadequate intake is capable of causing a low serum concentration, whence megaloblastic anaemia is still a long way off 2 Most patients receiving long-term PAS therapy are given at some stage various roborants in which vitamin B_{12} is frequently a component Although the absorption is reduced it may be compensated by an above normal intake, which prevents the development of a vitamin B_{12} deficiency sufficient to cause megaloblastic anaemia. 3 Since megaloblastic anaemia is not known to be a complication of PAS therapy, its possible presence has not been taken into consideration, and it therefore has evaded diagnosis or has been diagnosed as Addisonian pernicious or tapeworm pernicious anaemia It should be emphasized that careful examination — using the Schilling tests as an aid — will make it possible to differentiate the megaloblastic anaemia caused by PAS from the Addisonian pernicious anaemia or from that due to *Dibothriocephalus latus*, and that this differential diagnosis is important from the viewpoint of treatment of the anaemia

Summary

Nineteen of 21 patients on long term PAS therapy had a clearly lower Schilling test value during treatment than when the patients were without PAS The addition of IF did not improve the Schilling values during treatment A single dose of PAS given in connection with the Schilling test had no effect on the values, and the development of a disturbed vitamin B_{12} absorption re-

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Fatal Hypoglycaemia Associated with Retroperitoneal Tumour

By

T E Næss-Schmidt, S Jarnum, K Jørgensen and F Koch

Extrapancreatic tumours associated with fatal or recurrent hypoglycaemia have been reported in about 65 cases Extensive analyses of extracts of the tumours to demonstrate the presence of insulin or insulinlike activity have been carried out in several instances However the results are controversial and suggest the existence of several species of tumours with different physiological and biochemical properties

The present report deals with one such case who died in hypoglycaemic coma The tumour was located in the retroperitoneal space It was subjected to a number of post mortem analyses including immuno assay chemical analysis and biological testing

Case report

The patient was a 56-year-old male. In July 1962 he was admitted to a medical department because of growing oedema of the legs over a period of 8 days and complete urinary incontinence for 2 days. Except for periodic paraesthesia in both legs within the previous 6 months his case history was non contributory

On admission a cauda equina syndrome was present Radiograms of the spine and pelvic bones were normal He was suspected to suffer from a growth in the spinal medulla and was transferred to a neurosurgical department A new radiogram of the spine was taken, and this time a destruction of the arch of the third lumbar vertebra was found to be present. At suboccipital myelography a block was found almost at the same site.

An operation was performed *Laminectomy* was made of the fourth, third and part of the second lumbar vertebrae The arch of the third one was rumbling because of a tumour which penetrated deeply *into* the vertebral body The tumour was removed.

At freeze microscopy the tissue resembled myeloma However this diagnosis was changed after paraffin embedding The tumour tissue consisted of columns and nests of tumour cells with an abundant cytoplasm and round often excentric nuclei Staining for mucus was positive for a substance between the tumour cells The *appearance* of the tumour *resembled that of a tumour* of pancreatic island tissue Therefore special *stainings were performed to* demonstrate beta-granules *in* the cells, but they failed to do so which might be *expected of the*

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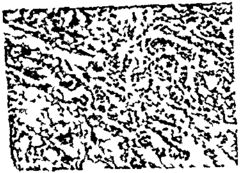


Fig 1 Operation specimen

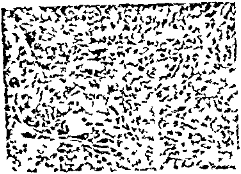


Fig 2 Autopsy specimen

Blood chemistry is listed in table 1 The main findings included a severe hypoglycaemia (blood sugar 19 mg/100 ml) a marked metabolic acidosis with extreme hyperkalaemia (8 mEq/l) and moderate uraemia Serum protein and sodium were elevated haemoglobin slightly depressed

Treatment and course As soon as the presence of hypoglycaemia was established glucose was given intravenously in a total amount of 75 g Minor muscle contractions in the extremities and sharp blink reflexes followed as the only response the coma remaining quite unchanged In addition he received intravenous infusion of Macrodex and serum and later blood because of the blood pressure and melaena in the rectum However following a transient improvement his condition rapidly deteriorated He died 10 hours after admission

Autopsy findings At autopsy 20 hours *post mortem* a large retroperitoneal tumour was found located mainly to the left of the lumbar spine It penetrated deeply into the posterior wall of the abdomen over a large area and anteriorly it protruded as a rounded 10 by 10 cm big partially encapsulated mass On section it consisted of white hard areas separated by yellowish soft necrotizing tissue

It was impossible to remove the whole tumour Its weight was roughly estimated to be about 1 000 g

There were no lymph node metastases

Severe pulmonary oedema was present Section of the liver showed a nutmeg liver

In the rectal ampulla was found a small melaena but nowhere else was there evidence of gastrointestinal bleeding and no source of bleeding could be detected

Spleen kidney adrenals pancreas and brains were all normal

Microscopical examination of the tumour Microscopical study of the tumour showed a somewhat lobulated neoplastic tissue The individual lobuli were separated by a tiny connective tissue framework and consisted of a diffuse arrangement of medium-sized to large polyhedral cells The nuclei varied markedly in size and shape and were most often lightly stained with an irregular chromatin structure and prominent pointed nucleoli Few mitotic figures were observed In many of the cells the eosinophilic cytoplasm was granulated and vacuolated giving the cells a resemblance to lipoblasts A small part of the tumour was necrotic and small areas of haemorrhage were noted Staining for mucus and reticulin was negative Special staining procedures for pancreatic island tissue as aldehyde fuchsin aniline-orange and chrome allun were also negative

Unfortunately the specimen was embedded in paraffin before the nature of the tumour was elucidated Thus the opportunity to make special stainings for lipoid substances was missed M D Liposarcoma.

Microscopy of adrenal glands and pancreas failed to reveal anything abnormal

Examination of tumour extract A lump of 117 g of tumour tissue was saved and frozen

TABLE I. Laboratory findings at admission. 75 g glucose was given intravenously at 7 a. m.

	7 a. m.	10 a. m.	12 a. m.
Haemoglobin	13 g/100 ml		
Serum protein	8.2 g/100 ml		
Serum chloride	108 mEq/l		
Standard bicarbonate	9.5 mEq/l		
Serum sodium	166 mEq/l	152 mEq/l	
Serum potassium	8.0 mEq/l	7.6 mEq/l	
Serum creatinine	2.4 mg/100 ml		
Blood urea	64 mg/100 ml		
Blood sugar	19 mg/100 ml	200 mg/100 ml	60 mg/100 ml
Serum barbiturate	0.5 mg/100 ml		
Blood ammonia	1.4 µg/ml		
Serum bilirubin	1.1 mg/100 ml		
Arterial blood 7 a. m.	Oxygen saturation	87 %	
	pH	7.13	
	Standard bicarbonate	8.0 mEq/l	
	pCO_2	21 mm Hg	

formalin fixed material. M. D.: Hormone-producing carcinoma probably of pancreatic origin (sign. E. Christensen).

The postoperative course was uneventful. There was still a moderate paresis of the lower extremities. He was treated with calciferol and X-ray irradiation of the lumbar spine. A primary tumour was thoroughly, but unsuccessfully sought for. Radiographs of the chest, pelvic bones, hip joints, colon, stomach and a small intestinal series were all normal as was intravenous pyelography. Paper electrophoresis and immuno-electrophoresis of serum revealed no abnormal proteins. Subsequently he recovered further. He was discharged in a fair condition 3 months after admission.

At home he carried on well and presented no new symptoms until the final admission 11 months later, on Sept. 6, 1963. The day before he felt weak and nauseated and started copious vomiting. In the evening he had a barbiturate injection intramuscularly and fell asleep. At 4 a. m. his wife woke up, because his respiration was deep and stertorous. She was unable to wake him up, and he was immediately admitted to hospital.

Physical examination. On arrival he was comatose, with sallow face and peripheral cyanosis. His body temperature was 36.9° C, pulse regular, 148 per minute, blood pressure 60 mm Hg. His respiration was deep and slightly accelerated (18 per minute). Skin and tongue were dry, cutaneous turgor diminished. He had no stiff neck or back. The pupils were contracted, light reflexes normal. Physical examination of heart and lungs was normal. The liver was felt 2 cm below the right costal margin, the bladder 10 cm above the symphysis. No evidence of ascites was present. The lower extremities were atonic with absent reflexes.

A rectal examination revealed greyish black stools which gave a strongly positive benzidine reaction.

Laboratory examination. The bladder was emptied by catheterization. It contained 1000 ml urine without sugar or protein.

An ECG showed high, tent shaped T waves in the second extremity lead suggestive of hyperkalaemia.

Table II summarizes the results given above and those of similar investigations reported by others

Discussion

At admission in coma the patient displayed several biochemical findings which apparently did not fit each other. The essential disturbance seemed to be severe hypoglycaemia (blood sugar 19 mg/100 ml), but at the same time a marked metabolic acidosis (standard bicarbonate 8 mEq/l) existed, which was far from compensated in spite of an obvious hyperventilation (p CO_2 21 mm Hg). The acidosis could at least partially be responsible for the hyperkalaemia present. Uraemia and diabetic ketoacidosis could be excluded as could poisoning (e. g.) methanol) for lack of anamnestic support. Copious vomiting in the preceding 20 hours explained his state of dehydration but not the electrolyte disturbances.

It is possible that excessive glucose consumption by the tumour brought about the acidosis. This would explain both hypoglycaemia and acidosis due to lactic acid formation during anaerobic glycolysis. An elevated lactic acid concentration in venous blood from patients with similar tumours has been reported (2 4). The hypothesis that the tumour consumes large amounts of glucose has been suggested by Sellman *et al* (17).

The first detailled description of the *pathological lesions* was given by Staffieri *et al* (19). In a large retroperitonal tumour they found 2 types of cells on microscopy round cells containing reticulin and spindle-shaped cells without reticulin. The tumour had been removed surgically. The hypoglycaemic attacks ceased completely following the operation.

Staining after Gomori's method produced blue granules in a few round cells similar to beta-cells of the pancreas in a tumour reported by Skillern *et al* (18). The authors believed that these tumours consist of atypical Langerhans' cells with insulin activity and a low grade malignancy, although they failed to recover insulin or insulin like activity from the tumour in their own case. Liver metastases have been reported in two cases (7, 8), and in a third case local recurrence was observed 7 years after extirpation (1).

A histological diagnosis of liposarcoma was made in the present case and in the one described by Hines (7).

The present case is the first reported which comprises two microscopical examinations of the tumour at a one year interval. The microscopical diagnoses made on the two occasions were — as already mentioned — not identical, but they are not incompatible.

The microscopical picture of a liposarcoma found at autopsy might be the morphological expression of a de-differentiation of the surgically removed epithelial tumour. This does not imply that the epithelial tumour was converted to a mesenchymal one but only that the tumour found at autopsy was microscopically indistinguishable from a liposarcoma. This point of view is supported by a great resemblance of the most de-differentiated areas in the first tumour to the most differentiated areas in the second one.

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Since the primary purpose of the acid ethanol extraction was to establish whether the insulin content was high enough for the isolation of crystalline insulin, it had been necessary to purify the alcoholic extract in such a way that a possible content of insulin like activity in the tumour would be diminished to a significant degree during the preparation of the aqueous solution. Consequently the given figure, 2 milliunits per g, presumably makes an underestimation of the actual content of insulin like activity in the tumour

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tained a dialysable glucose 6-phosphate inhibitor

Finally, it cannot be excluded that the tumour produces a sub tance which directly or indirectly stimulates the secretion of insulin in the pancreatic islar tissue itself In this connection it is interesting that experiments have been reported which suggest the existence of a hormone like substance that stimulates pancreatic insulin production, when glucose is instilled into the jejunum (10)

The *therapy* should be radical extirpation of the tumour whenever possible If not, only palliative treatment can be offered Besides glucose administration, glucagon may have a distinct beneficial effect (13 22)

Summary

A case of fatal hypoglycaemia caused by a large retroperitoneal tumour is reported Histologically the tumour found at autopsy could be characterized as a liposarcoma One year previously the patient had a tumour removed from the same site at the lumbar spine By the time of operation only neurological symptoms and no hypoglycaemia were present The operation specimen resembled that of a tumour from pancreatic islar tissue histologically It is suggested that the tumour later de-differentiated to a tumour which although of epithelial origin, microscopically resembled a liposarcoma

By immuno assay rat diaphragm and fat pad technique the insulin like activity in an aqueous solution prepared by purification of an acid ethanol extract, was found to equal at least 2 milliunits of insulin per gram of tumour

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Objections could be made that the lack of island-like tissue in the autopsy specimen could be due to the fact that only a small part of the tumour was subjected to histological examination. It is true that most of the tumour was saved for chemical analysis, but it is unlikely that the innumerable sections performed (more than 200) should be without such tissue if present at all.

The clinical course of the case was remarkable. At the time of operation pancreatic island tissue was present but no clinical hypoglycaemia. One year later no islar tissue could be demonstrated but nevertheless the patient died from hypoglycaemia. It seems as if it was the de-differentiated tumour cells which have the ability to provoke hypoglycaemia in one way or another.

Hypoglycaemia can be produced by other types of tumours as well. Tranquada *et al.* (20) reported an extraordinary case in whom hypoglycaemia was produced by a large adenocarcinoma of the caecum.

Tumour content of insulin or insulin like activity

The pathogenetic relationship between tumour and episodes of hypoglycaemia is well established, because the symptoms subside when the tumour is removed (11, 14, 18).

No insulin or insulin like activity was found in 4 patients with hypoglycaemia and peritoneal pseudomyxoma (*16*) and in one with intra-abdominal mesothelial cell sarcoma (17). The latter group conceived that hypoglycaemia was produced by excessive glucose uptake by the tumour, since it contained large amounts of aminoacids and 1,5 g glycose per 100 g.

In several instances insulin or insulin like activity has been demonstrated. The concentrations found have varied considerably, the maximum values being 0.5—1 *unit of insulin per g of tumour* tissue (2, 4). Usually the concentration has been much lower and of the same order of magnitude as in the present case. Although about 100 times higher than the normal plasma insulin level, *the value found in the present case* (about 2 milliunits per g) does not seem exceedingly high. However, the insulin *content* is not necessarily a reflection of insulin *production* by the tumour. It may well be that part of the "malignant" feature of the tumour cells is a lack of ability to store insulin in the same way as in the beta cells of the normal pancreas. This would explain — at least partially — the discrepancy between the low insulin concentration in the tumour and its profound effect on the blood sugar level.

As a third mechanism one must consider the possibility that the tumour tissue produces hitherto unknown compounds which deeply interfere with glucose metabolism, and in a way which only in some respects resembles that of insulin. It is supported by recent findings by Perkoff and Simons (15). From a hypoglycaemia-inducing tumour they isolated a heat stable, non dialysable *substance*, which accelerated both glucose uptake (like insulin) and glycogenolysis (contrary to insulin) by the isolated rat diaphragm. Immunologically, the substance was not identifiable as insulin. In addition this tumour con

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From the Medical Clinic (Head N Törnblom M D), Umeå Lasarett,
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Administration of Orto,para'-DDD to Diabetics with Vascular Changes[1]

By

Nils Törnblom and Pontus Wiklund

Tornblom (37, 38) previously reported the effect of DDD administration on diabetics with hyaline vascular changes and hyperpolysaccharidemia The administration of DDD to diabetics was intended partly to reduce the adrenal function and partly to decrease the polysaccharide content in the serum

These therapeutic tests began in 1954 The following clinical and experimental observations were then presented in support of the concept that the hyaline vascular changes are influenced by pituitary adrenal function 1) Poulsen s ca e of diabetic retinopathy where the retinopathy disappeared when the patient developed a Sheehan s syndrome 2) The reports of the favorable effects of adrenalectomy or hypophysectomy in diabetics with vascular changes 3) The reports of the increased weight of the adrenals and a higher incidence of adrenocortical adenomata in diabetics with Kimmelstiel Wilson s syndrome 4) Hyaline changes in glomeruli similar to those in diabetic glomerulosclerosis induced in rabbits by administering cortisone or ACTH 5) The increased secretion of corticosteroids in diabetic acidosis

In 1949 McManus (26) suggested that the hyaline material of the glomerular lesions in diabetic nephropathy might originate from the polysaccharides of the blood This hypothesis has already been discussed by Tornblom (35, 38)

By administering DDD to diabetics it was possible to influence the adrenal function so that the effectiveness of ACTH in stimulating the latter decreased Moreover, the hyperpolysaccharidemia was reduced The question as to whether the development of the hyaline vascular changes was affected was left unanswered

It is not the intention of the authors here to enlarge upon our knowledge within this sphere With regard to McManus's supposition that the hyaline

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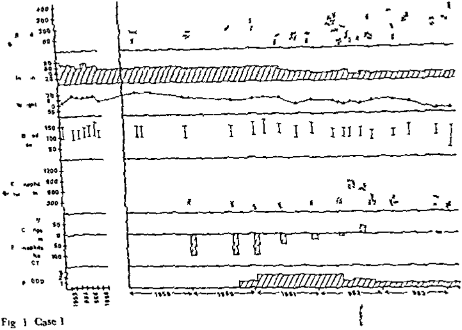


Fig 1 Case I

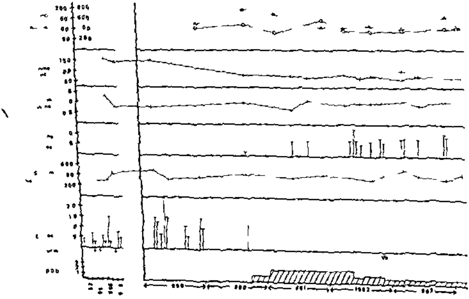


Fig 2 Case I

TABLE I Case 1 Diabetic retinopathy and nephropathy diagnozed since 1951

		Sight		
		Right eye		*Left eye*
1951		0.9–1.0		1.0
1953		1.0		1.0
1954		0.9–1.0		1.0
1955		1.0		1.0
1957		0.9		1.0
1959	FM	2 m		1.0
		0.2		1.0
1960	FM	4 m		1.0
1961	FM	4 m		1.0
	Hardly	0.1		0.9
1962		4/60		0.9
		1/60	Almost	1.0
		2/60	Almost	1.0
	Hardly	0.1		1.0
1963	Hardly	0.1		0.9–1.0
	FM	1 m		0.9–1.0
		0.1–0.2		1.0
1964		0.1		1.0

FM = Finger movements

vascular changes are due to the precipitation of glucoproteins in the blood, we would point out that this is considered to be of no current interest (33). Odin and Tornblom (29, 31) have shown by a direct chemical analysis that the hyalinized glomeruli from diabetics do not contain more carbohydrates than normal glomeruli and normal blood proteins and that, unlike normal glomeruli, they contain blood proteins.

During the years 1957 and 1958 Nichols and Hennigar and also Cueto and Brown (6, 7, 8, 30) established that the adrenolytic component in DDD consisted of the isomer orto, para'-DDD. The latter was isolated by Cueto and Brown. We considered it of interest and justifiable to continue the treatment of diabetics with this more effective substance. At that time o,p'-DDD was not available on the market. Accordingly contact was established with Pharmacia Ltd., Uppsala to make possible the production of this substance. Dr. Mårtenson, of Pharmacia Ltd., succeeded during the period 1958—1959 in synthesizing o,p'-DDD. Dr. Brown placed samples of his substance at our disposal and the identity of his and the produced substance was established. The toxicity assessments (Dr. Hint) on albino mice weighing from 17 to 28 g revealed with a single administration of the substance $LD_{50} > 3$ g/kg. The observation period was 2 weeks.

For the therapeutic treatment with o,p'-DDD the diabetic patients were chosen so as to be similar in type to those previously treated with DDD. The patients were examined and followed up in a similar manner. The amount of polysaccharides in the serum was not, however, investigated. When the administration of o,p'-DDD was commenced nothing was known of its effects on humans. Consequently relatively small doses were administered.

So far o,p'-DDD has been administered to 11 diabetic patients with vascular changes. During the treatment one of the patients developed a typical Addison's syndrome. In connection with this the patient's insulin requirement diminished considerably. This patient was the one who received the largest amount of o,p'-DDD. Her case report will be given in detail. The remaining patients will be briefly dealt with.



Fig 3 Case 1 April 1962

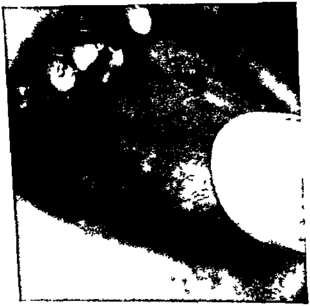


Fig 4 Case 1 February 1964

Case reports

Case 1 Woman, born in 1936 Childless Diabetes since 2 years of age Diabetic retinopathy and nephropathy known since 1951 (table I) She has been on an *ordinary* diet for diabetics From the end of 1960 she was given a daily dose of 20 mg o,p -DDD per kg body weight (figs 1 and 2) The substance was administered orally The patient's diet was not changed At the beginning of 1961 the dose was doubled ACTH-tests were performed with intravenous infusions of ACTH During 1960 this administration brought about a considerable reduction of the number of eosinophil granulocytes in the blood In the middle of 1962 the patient had troublesome attacks of hypoglycemia The supply of insulin, already previously reduced, was further reduced The pre test values of the eosinophil granulocytes rose and their number was now scarcely affected by the administration of ACTH The patient developed skin and mucous membrane pigmentations of the type seen in Addison's disease Fig 3 shows the pigmentations of the oral mucous membrane The amount of alkaline phosphatases in the blood had increased during o p -DDD administration The dose was then reduced The pigmentation of the oral mucous membrane revealed a tendency to diminish, but subsequently returned (fig 4) The inulin and creatinine clearance values have, during the 3 1/2 years' administration of o,p DDD, remained essentially unchanged

The patient had, during the period 1951 to 1960 a persistent proteinuria with Esbach-values varying from 0 025 to 0 3 % During 1961 her proteinuria almost vanished and since then her urine has off and on been free of protein Her eyeground changes have hardly altered during the treatment with o,p DDD The visual acuity of the one eye had increased (table I) She was sterilized in 1961 and in association with this she had a transitional icterus During the past year she has had abdominal trouble the type of which could not be established (abdominal angina?) She has been examined with regard to the amount of urinary 17 keto and 17 hydroxycorticosteroids before and during ACTH administration For technical or other reasons the values have been uneven and no clear change has been established In Feb 1964 the amount of aldosterone in a 24-hour quantity of urine was 2 7 μg (low normal value)

The patient's general condition is good She was married in 1963 She is working as a clerk

This patient thus developed an Addison's syndrome during the administration of o,p DDD In association with this the insulin requirement diminished considerably She *has not received any substitution treatment* in connection with the reduced adrenal funtion The amount of alkaline phosphatases in the serum has been somewhat high, otherwise no signs of liver injury have been observed *The disappearance of a pronounced* proteinuria known to have existed for 10 years, in association with the administration of o,p -DDD is remarkable

Cases 2 to 11 In three of these ten cases the treatment was discontinued, in two cases due to removal of the patients from the district, in the third case owing to the patient having a number of uncharacteristic troubles, tingles and swollen eyelids, which the patient considered due to the treatment and which made him decide to discontinue it In these three cases the preparation was given in smaller doses than 40 mg/kg/day, or in this dose only during a short time No definite signs of a reduced adrenal function were observed

Four patients died during or after certain medication, all of them in uremia In these cases the patient showed signs of renal insufficiency in the form of raised serum creatinine or urea nitrogen values before the medication commenced The o,p DDD dose was less than 40 mg, or this dose was given during a short time One of the patients however, received 40 mg for about 12 months In three of these four cases autopsy was performed In no case were there clinical or patho anatomical signs of adrenal lesions In the autopsy cases the kidneys revealed glomerulosclerosis of the Kimmelstiel Wilson

type or glomerulosclerosis and pyelonephritis.

In the three remaining cases the dose of o p DDD was less than 40 mg or the dose was given for a short time. Essentially token treatment is now being given to one case — a patient who had moved to a distant school for the blind. The patient did not wish to discontinue the treatment.

Discussion

The administration of DDD or o p - DDD produces in animals changes of the zona reticularis and zona fasciculata in the adrenal cortex and reduces the production of corticosteroids The zona glomerulosa and the aldosterone production of the adrenal cortex are influenced to a less degree (3, 4 30 34) Brown (5) found that the administration of o p DDD to humans reduced the amount of corticosteroids in the blood Bergenstal et al (3) and others (15 40) have treated patients with metastasizing adrenal cortical carcinoma with o p DDD and in no small number of patients have achieved temporary success For a period of months the patients were given up to 10 g of o p DDD per day The amount of 17 hydroxycorticosteroids and 17 ketosteroids in the urine usually diminished At the same time the patients were given prednisone or similar substances

It is therefore not surprising that — as in the case of the previously described patient — the administration of o p DDD may produce a clinical picture of Mb Addison Accordingly it is quite natural that the diabetic patient s requirement for insulin diminishes considerably The fact that it was not necessary to substitute the adrenal function may be explained by the inconsiderable influence of the substance upon the zona glomerulosa and aldosterone production As regards toxic effects we have discerned only a certain increase of the alkaline phosphatases in the serum. This increase is considered to be an expression of a liver toxic effect In our opinion it was not such as to warrant discontinuation of the treatment

The aim of the treatment was to ascertain if it influenced the course of the so-called diabetic vascular changes The fact that in the diabetic patient who developed an Addison s syndrome, there did not arise during as long as 3 1/2 years any demonstrable progress of diabetic retinopathy, or a reduction of inulin or creatinine-clearance is really remarkable This does not enable it to be decided whether or not there are effects upon the vascular changes throughout the course That a pronounced proteinuria registered during the past 10 years almost disppeared and periodically disappeared completely is, however, much more remarkable

The effects of surgical adrenalectomy on a diabetic patient with retinopathy and proteinuria have been studied by Green et al (13) The adrenal function was replaced by the administration of adrenal cortex extract (Upjohn Co) and intermittently by DOCA The proteinuria diminished A favorable effect of adrenalectomy on proteinuria in some patients has also been reported by Wortham and Headstream (41, 42), Martin and Wilson (26), Malins (23) and Graef (11) These investigators replaced the adrenal function by administering cortisone or similar substances

One of the patients, the one who received the greatest amount of o,p DDD, developed a typical Addison's syndrome during treatment In association with this the patient s requirement for insulin diminished considerably It was not necessary to replace the adrenal function The treatment covered a period of 3 1/2 years During the period in question the patient's retinopathy did not progress and the renal clearance values did not decrease During the ten years previous to treatment the patient had a pronounced proteinuria The latter disappeared almost entirely when the patient developed signs of adrenal insufficiency

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Martin and Wilson report that the proteinuria decreased *when the patient* had a mild adrenal insufficiency. Graef and Mailer (12), Malins (24) and Oosterhuis et al. (32) made no mention of a possible change of proteinuria in association with the surgical intervention. Martin and Wilson discuss the works of other authors concerning the effects of adrenalectomy or hypophysectomy, or of *inter alia* cortisone and testosterone administered to rats with proteinuria produced by renin administration, or to certain male rats with spontaneous proteinuria.

A favorable effect of hypophysectomy upon proteinuria in some diabetic patients with retinopathy and proteinuria has been reported by Luft and Olivecrona (19), Kinsell et al. (16), Luft et al. (21), Hernberg et al. (14), Vannas et al. (39), Ainslie et al. (1) and Lundbäck et al. (22). In isolated cases *the proteinuria disappeared completely*. The substitution treatment consisted of cortisone and thyroid preparations and in some cases of different types of sex hormones. Lawrence et al. (18), who through radiation treatment reduced the function of the hypophysis in diabetics with retinopathy, mention nothing of the effects upon a possible proteinuria.

We are of the opinion that the regress of the proteinuria in the patient previously described by us is not coincidental, but is probably due to the reduced function of the adrenal cortex induced by the administration of o,p'-DDD. It is hardly worth while discussing how this effect has arisen, as our knowledge of the genesis of long term diabetic complications is so incomplete.

In this connection we would like to point out certain observations made by our group in Umeå (17, 27, 28). The long-term diabetic complications of the vascular type are generally considered to be progressive or possibly stationary. Melin has studied a common long term diabetic complication, previously undescribed, where the course is quite different. This complication manifests itself in the form of small, rounded, brown, atrophic, circumscribed skin lesions located on the lower extremities. They disappear after one or more years. In the meanwhile new changes in the skin arise whereby the total picture progresses or remains unchanged. They are characterized histologically as local atrophy of the epidermis. Microangiography reveals that dermis corresponding to these lesions contains an increased number of blood-vessels. In all probability these skin lesions are not traumatic. A fluorescence microscopic examination of these skin lesions by means of anti human gammaglobulin reveals a fluorescence similar to that previously described for skin from patients with discoid and disseminated lupus. The fact that the hyalinized renal glomeruli in diabetes contain gammaglobulin has previously been described by Odin and Törnblom 1959 and by Freedman et al. 1960, 1962 (9, 10, 29, 31).

Summary

Eleven diabetic patients with diabetic retinopathy and other signs of vascular changes have received orto, para'-DDD with a view to reducing the adrenal cortical function.

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From the Department of Medicine (Head Sven Johnsson M D) and the Department of Clinical Chemistry (Head Bertil von von Porat M D) Central Hospital Sundsvall, Sweden

The Diagnostic Value of Protein-bound Radio-iodine Estimation in an Endemic Goitre Region

By

Ove Lundvall Sven Johnsson and Bertil von Porat

The introduction of the radioactive iodine test was a considerable advance in the diagnosis of thyroid dysfunction It has been found however that radio-iodine tests based on determination of thyroid uptake or urinary excretion frequently provide misleading information in endemic goitre areas (10) In these areas persons without any signs of hyperthyroidism often show a raised tracer-iodine uptake and a lowered tracer iodine excretion simulating hyperthyroidism

Even though endemic goitre may be caused by goitrogens (6) the most important factor is considered to be iodine deficiency (1 10 18 28) In iodine deficiency one finds a low inorganic plasma iodine level yet as a rule a normal absolute iodine uptake in the thyroid (1) Absolute iodine uptake is maintained at a normal level by increased thyroid iodine clearance which explains the increased uptake of the tracer iodine (1, 15)

There are now many methods available to establish whether a raised tracer-iodine (^{131}I) uptake is due to hyperthyroidism or to iodine deficiency

In the triiodothyronine test (31) the patient is given 100 μg of triiodothyronine a day for a week, by which time the value obtained by the ^{131}I test will be normal if the increase is caused by iodine deficiency, but not if it is due to hyperthyroidism

Studies of the ^{131}I uptake curve were used to differentiate between iodine deficiency and hyperthyroidism (16) The maximum uptake *is as a rule reached* earlier in hyperthyroidism than in iodine deficiency In hyperthyroidism it is usually followed by quicker reduction of the thyroid *radioactivity* than in iodine deficiency However, Eichhorn (9) found in an endemic goitre area that the uptake curves show rises in euthyroids quite like those in hyper*thyroids* He also found that the radioactivity in the thyroid did not decrease

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Th_2 20—30 % Th_{24} 23—55 % U_{24} 30—60 %

Normal value for PBI is 3 8—8 μg%

Determination of total plasma radio activity (total ^{131}I) and $PB^{131}I$ were made with an apparatus supplied by Abbott & Co Total ^{131}I and $PB^{131}I$ were measured in a well counter with the same equipment as used for the tracer dose determinations (10) The total ^{131}I sample was passed through an ion exchange column (Iores n Abbott or Dowex 1 X8 50—100 mesh) which removed the free ^{131}I leaving the $PB^{131}I$ in the sample (33)

Our method has given a normal upper limit for $PBI^{131}I_{24}$ of 0 27 % of the administered dose per litre plasma Several authors give the normal upper l mit for $PB^{131}I_{48}$ as 0 40 % per dose per litre plasma (13 27 29) Since our control group is small we have chosen this value

Conversion ratio (C R) is obtained by $\frac{PB^{131}I_{24}}{total\ ^{131}I_{24}} \times 100$ (%) Clark et al (5) who introduced the C R for the estimation of thyroid funct on found it normally to be between 13 and 42 % (average 27 5 %) The upper normal l mit is considered to be 35 % (32)

The index 48 hours ($Index_{48}$) after the administered dose is obtained by $\frac{PB^{131}I_{48}}{total\ ^{131}I_2}$ The upper normal l mit is suggested by Eichhorn to be 0 2 None of the patients with hyperthyroidism had lower values whereas in those with an index above 0 2 the probab l ty that the patient was euthyro d was only about 6 % (9)

The 24 hour index ($Index_{24}$) for the dosage given is obta ned by $\frac{PB^{131}I_{24}}{total\ ^{131}I_2}$ From the above given values for $Index_{48}$ and $PB^{131}I$ at 24 and 48 hours the normal upper level for Index 24 is calculated $\frac{0\ 27 \times 0\ 2}{0\ 4} = 0\ 135\ (0\ 14)$

Material

The series is composed of two groups

A "Iod ne deficiency group 41 cl nically euthyro d cases with raised ^{131}I uptake in the thyroid and lowered ^{131}I-excretion in the urine but normal PBI values Nine cases had diffuse goitre and 26 cases nodular goitre Six cases had little or no thyroid enlargement The group is composed of 2 men and 39 women with ages ranging from 18 to 72 years (average 48)

B Hyperthyroid group 80 clinically hyperthyroid cases with raised ^{131}I uptake and lowered ^{131}I excretion and raised PBI values Twenty eight cases had diffuse goitre and 28 cases nodular goitre Twenty four cases had little or no thyroid enlargement The group is composed of 15 men and 65 women — ages ranging from 14 to 82 years (average 51 years)

Cases which have been medically radiologically or surgically treated for goitre have not been included in any of the groups

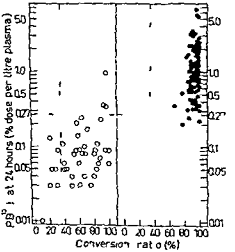


Fig 1 The distribution of $PB^{131}I$ (logarithmic scale) at 24 hours and C R in 41 "iodine-deficiency cases (○) and in 80 hyperthyroid cases (●) (Note that the convers on rat o is repeated on the abscissa)

Results

PROTEIN BOUND RADIOACTIVE IODINE AT 24 HOURS

$PB^{131}I$ was determined at 24 hours throughout the series The results are shown in fig 1

so much quicker in hyperthyroid cases than in euthyroid cases as to permit of any conclusions about the functional state of the thyroid

The thiocyanate suppression test (23) has recently been introduced to differentiate between hyperthyroid patients and euthyroid patients with a high ^{131}I-uptake *Thiocyanate is given for two* days to a total dose of 3 g after which the ^{131}I-test is repeated In the euthyroids the 24-hour uptake dropped to below 20 % The reduction of the 24 hour uptake in hyperthyroid patients was less and the 24 hour uptake figure was always above 20 % This test appears useful, but requires further investigation for more conclusive evaluation

In hyperthyroidism the absolute iodine uptake is considerably increased but in iodine deficiency *it is normal or slightly* lowered (1, 15) Absolute iodine uptake in the thyroid is estimated by measurement of ^{131}I-uptake and determination of ^{131}I-activity and inorganic stable iodine in the plasma Estimation of inorganic iodine in plasma is difficult and time consuming and thus the method is hardly suitable for clinical use

Another possibility is to correct the *iodine deficiency* by administration of iodine and then to repeat the ^{131}I-test We have tried the iodine repletion test of Burrel and Frazer (10) but 7 % of the euthyroid patients developed hyperthyroidism during the course of the test For this reason the test was abandoned

Our hospital is situated in an endemic goitre area in which euthyroid patients often have increased ^{131}I-uptake values and decreased ^{131}I excretion values (10) In order to find a more reliable measure of the functional state of the thyroid we have determined plasma protein bound radio-iodine (PB^{131}I), conversion ratio *(C R)* and *Eichhorn's index (Index)*

A measure of thyroid-hormone secretion is obtained by the estimation of PB^{131}I in plasma at various intervals after administration of a radioiodine dose For clinical purposes *PB^{131}I* is generally estimated at 48 or 72 hours

The conversion ratio is arrived at by estimating the percentage of total radio activity in plasma which is protein bound at a given time For clinical purposes this is usually done 24 hours after administration of the tracer dose

Eichhorn (9) has developed a modification of Blondal's index (4) He estimates the quotient between protein bound radio-iodine in plasma at 48 hours and total radio iodine activity in plasma at 2 hours (Blondal did not estimate the protein-bound fraction but simply the quotient from total radioactivity in plasma at 48 and 2 hours)

The purpose of this investigation is to judge the usefulness of the PB^{131}I estimations, the conversion ratio and Eichhorn's index in an endemic goitre area

In about half the cases we have determined both the PB^{131}I and Eichhorn's index at 24 and at 48 hours in order to assess the comparability of these estimations

Methods

Methods for tracer dose test with ^{131}I, protein bound iodine (PBI) and basal metabolic rate (BMR) are given in a previous paper (10) Normal values for the 2 hour (Th_2) and 24-hour (Th_{24}) uptakes in the thyroid and for urine output in 24 hours (U_{24}) are

In the three cases where $PB^{131}I_{24}$ was raised the C R was 95 % or more

B Hyperthyroid group (80 cases)

In the hyperthyroid group the C R was above 35 % in all cases and the lowest value was 67 % Only 3 in the hyperthyroid group had a C R below 80 % On the other hand 14 'iodine-deficiency' cases (34 %) had a C R of 80 % or more The 3 hyperthyroid cases with normal $PB^{131}I_{24}$ had C R of 77 %, 86 % and 100 % respectively

PROTEIN BOUND RADIOACTIVE IODINE AT 48 HOURS

$PB^{131}I$ was estimated both at 24 and 48 hours in about half of the series (19 'iodine deficiency' cases and 38 hyperthyroid cases) The relationship between $PB^{131}I_{24}$ and $PB^{131}I_{48}$ is shown in fig 2

1 The 'iodine deficiency' cases (19 cases)

In the 19 'iodine deficiency' cases the $PB^{131}I_{48}$ was less than 0 40 % except in one case which had a value of 0 61 % All 19 cases had normal $PB^{131}I_{24}$ values

Data for the 'iodine deficiency' case with raised $PB^{131}I_{48}$ are given below

Case L F A 68-year old woman with a small nodular goitre who some months previously was suspected of suffering from hyperthyroidism Spontaneous remission Clinically euthyroid $PB^{131}I_{48}$ 0 61 %

Th_2 48 % Th_{24} 81 % U_{24} 12 % BMR ± 0 PBI 7 2 μg% $PB^{131}I_{24}$ 0 24 % C R 63 $Index_{24}$ 0 09 $Index_{48}$ 0 24

After iodine repletion test (according to Burrel and Frazer) a marked hyperthyroid state developed She was treated with 5 mC radio-iodine after which the toxic symptoms were alarmingly accentuated These were controlled by the administration of carbimazole

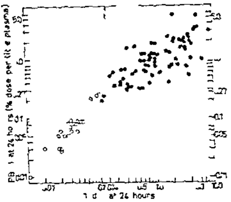


Fig 3 The relationship between $Index_{24}$ and $PB^{131}I_{24}$ (logarithmic scales) in 39 "iodine-deficiency" cases (○) and in 79 hyperthyroid cases (●)

B The hyperthyroid cases (38 cases)

In the 38 hyperthyroid cases the $PB^{131}I$ values at 48 hours were greater than 0 40 % except for one that had a value of 0 38 % In this case (S M) the $PB^{131}I_{24}$ was also normal Another case (L I) had a normal $PB^{131}I_{24}$ value In all the rest the $PB^{131}I_{24}$ values were elevated The estimation of $PB^{131}I$ at 48 hours thus showed one misleading value out of a total of 19 'iodine deficiency' cases and one out of the 38 hyperthyroid cases The estimation of $PB^{131}I$ at 24 hours showed no misleading values in these 19 'iodine deficiency' cases but 2 in the hyperthyroid cases

INDEX AT 24 HOURS

$Index_{24}$ was estimated in 39 "iodine deficiency" cases and in 79 hyperthyroid cases The relationship between $Index_{24}$ and $PB^{131}I_{24}$ is shown in fig 3

A The "iodine deficiency" cases (39 cases)

In the 39 "iodine-deficiency" cases the $Index_{24}$ was less than 0 14 in 36 cases

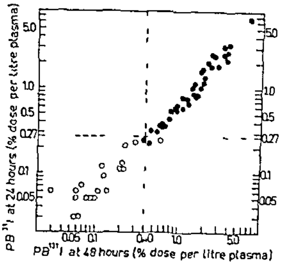


Fig 2 The relationship between PB^{131}I at 48 and at 24 hours (logarithmic scales) in 19 "iodine-deficiency" cases (○) and in 38 hyperthyroid cases (●)

A "Iodine deficiency" group (41 cases)

In this group the PB$^{131}I_{24}$ was less than 0 27 % in all cases except three whose values were 0 98 %, 0 36 % and 0 34 % respectively

A summary of data for these three cases follows

Case L. H A 43 year old woman clinically euthyroid, with an adenoma of about hen's egg size in the right lobe The scintigram showed a considerable uptake in the adenoma with only weak uptake in the otherwise slightly enlarged thyroid PB$^{131}I_{24}$ 0 98 %

Th_2 60 %, Th_{24} 67 %, U_{24} 19 %, BMR + 10 % PBI 5 8 μg%, C R 97 % $Index_{24}$ 0 73

Case I S A 28 year old woman clinically euthyroid with a moderately enlarged diffuse goitre PB$^{131}I_{24}$ 0 36 %

Th_2 49 % Th_{24} 66 % U_{24} 22 % BMR + 10 %, PBI 5 4 μg% C R 95 % $Index_{24}$ 0 21

Case S C A 27 year old woman clinically euthyroid with a moderately enlarged nodular goitre PB$^{131}I_{24}$ 0 34 %

Th_2 63 %, Th_{24} 80 %, U_{24} 11 % BMR + 2 %, PBI 7 2 %, C R 97 % $Index_{24}$ 0 24

B Hyperthyroid group (80 cases)

In this group the PB$^{131}I_{24}$ was more than 0 27 % in all but three cases These had values of 0 24 %, 0 22 % and 0 20 %

A summary of data for these three cases follows

Case S M A 82 year old woman with fairly severe hyperthyroidism and a very large multinodular goitre PB$^{131}I_{24}$ 0 24 %

Th_2 42 %, Th_{24} 63 %, U_{24} 10 %, BMR + 71 %, PBI 19 0 μg%, cholesterol 120 mg% C R 86 %, $Index_{24}$ 0 12, $Index_{48}$ 0 19 PB$^{131}I_{48}$ 0 38 %

Case L I A 48 year old woman with hyperthyroidism of moderate degree No distinct thyroid enlargement PB$^{131}I_{24}$ 0 22 %

Th_2 49 %, Th_{24} 71 %, U_{24} 20 % BMR + 93 %, PBI 10 6 μg%, cholesterol 163 mg% C R 100 %, $Index_{24}$ 0 17, $Index_{48}$ 0 34 PB$^{131}I_{48}$ 0 44 %

Case Ö K A 55 year old woman with hyperthyroidism of moderate degree and a moderately enlarged nodular goitre PB$^{131}I_{24}$ 0 20 %

Th_2 45 %, Th_{24} 66 %, U_{24} 23 % BMR + 40 % PBI 10 4 μg%, cholesterol 200 mg% C R 77 % $Index_{24}$ 0 11

Thus the PB$^{131}I_{24}$-determinations gave misleading values in 3 cases from each group or altogether 6 cases in 121 (5 %)

CONVERSION RATIO

C R was estimated in the entire series The results are shown in fig 1

A "Iodine deficiency" group (41 cases)

In the "iodine deficiency" group the C R was over 35 % in 34 cases (83 %)

TABLE I Comparison between $PB^{131}I_{24}$ and C R $PB^{131}I_{48}$ Index$_{24}$ and Index$_{48}$

Type of test	Iodine deficiency: No of cases	Iodine deficiency: No of misleading results	Hyperthyroidism: No of cases	Hyperthyroidism: No of misleading results	Iodine defic and hyperth: No of cases	Misleading results: No	Misleading results: %
C R	41	34	80	0	121	34	28
$PB^{131}I_{24}$		3		3		6	5
$PB^{131}I_{48}$	19	1	38	1	57	2	4
$PB^{131}I_{24}$		0		2		2	4
Index$_{24}$	39	3	79	2	118	5	4
$PB^{131}I_{24}$		3		3		6	5
Index$_{48}$	17	2	38	1	55	3	5
$PB^{131}I_{48}$		0		2		2	4

Discussion

Most workers have preferred to determine $PB^{131}I$ 48 or 72 hours after the administration of the tracer dose Protein bound activity is usually much greater at 48 or 72 hours than at 24 hours in hyperthyroidism In euthyroid cases, however the rise of $PB^{131}I$ activity after 24 hours is not so noticeable Thus estimation after 48 or 72 hours should give more accurate results than after 24 hours Staffurth (27) estimated $PB^{131}I$ at 24 and 48 hours in 25 cases $PB^{131}I$ was normal both at 24 and 48 hours in the 11 euthyroid cases and raised both at 24 and 48 hours in the 14 hyperthyroid cases In our series $PB^{131}I$ at 24 and 48 hours were compared in 19 'iodine-deficiency' cases and in 38 hyperthyroid cases In the 'iodine-deficiency' cases the $PB^{131}I$ values were all normal at 24 hours but in one instance it was raised at 48 hours This case however later developed into hyperthyroidism In the hyperthyroid cases $PB^{131}I$ was normal at 24 hours in 2 instances but normal in only one of those at 48 hours

The dignostic value of the $PB^{131}I$-determination has been shown by many workers (13 15, 17, 19, 26 27 29) It has however, been emphasized that the $PB^{131}I$ estimation gives uncertain results in cases of nodular goitre (27)

In iodine deficiency goitre there is generally raised iodine uptake and sometimes lowered intrathyroidal iodine pool (7) Both these factors may give a raised $PB^{131}I$ activity (3) There is thus reason to believe that the determination of $PB^{131}I$ will be more inaccurate in regions where goitre is endemic Barzelatto (2) found that $PB^{131}I_{24}$ was less than 0 45 % of a given dose in 95 % of a series of 83 euthyroid patients with endemic goitre

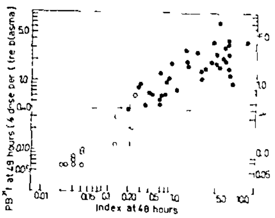


Fig 4 The relationship between $Index_{48}$ and $PB^{131}I_{48}$ (logarithmic scales) in 17 "iodine deficiency" cases (○) and in 38 hyperthyroid cases (●)

but above 0 14 in 3 cases (L H, Å S and S G) whose values were 0 73, 0 21 and 0 24 respectively These three also had raised $PB^{131}I_{24}$ whereas the other 36 were normal in this respect

B The hyperthyroid cases (79 cases)

In the hyperthyroid cases $Index_{24}$ was more than 0 14 in all but 2 instances (S M and Ö K) whose values were 0 12 and 0 11 They also had normal $PB^{131}I_{24}$ values Another of these 79 hyperthyroid cases (L I) had a normal $PB^{131}I_{24}$ value

Thus the estimation of $Index_{24}$ showed misleading values in 3 out of 39 "iodine-deficiency" cases and in 2 of the 79 hyperthyroid cases The estimation of $PB^{131}I_{24}$ gave misleading values in 3 of the 39 "iodine deficiency" cases and 3 of the 79 hyperthyroid cases

INDEX AT 48 HOURS

$Index_{48}$ was estimated in 17 "iodine-deficiency" cases and in 38 hyperthyroid cases The relationship between $Index_{48}$ and $PB^{131}I_{48}$ is shown in fig 4

A The "iodine-deficiency" cases (17 cases)

In the 17 "iodine-deficiency" cases the $Index_{48}$ was less than 0 20 in all but 2 instances These had values of 0 22 and 0 24 One of them (L I) had also a raised $PB^{131}I_{48}$ $PB^{131}I$ at 48 hours was normal in the other 16 "iodine deficiency" cases $PB^{131}I_{24}$ and $Index_{24}$ was normal in all the 17 "iodine deficiency" cases

B The hyperthyroid cases (38 cases)

In the hyperthyroid cases the $Index_{48}$ was more than 0 20 in all but one (S M) who had a value of 0 19 This case also had a normal $Index_{24}$ and normal $PB^{131}I$ values The remaining 37 hyperthyroid cases had raised $Index_{24}$ and $PB^{131}I_{48}$ whereas $PB^{131}I_{24}$ was normal in yet another case (L I)

Thus the estimation of $Index_{48}$ gave misleading values in 2 of 17 "iodine deficiency" cases and in one of 38 hyperthyroid cases The $PB^{131}I_{48}$-estimation produced misleading values in one of the 17 "iodine deficiency" cases and in one of the hyperthyroid cases The estimation of $Index_{24}$ in this part of the series showed no misleading value in the "iodine deficiency" cases but produced one in the hyperthyroid group The determination of $PB^{131}I_{24}$ in the same part of the series gave misleading values in none of the "iodine-deficiency" cases but in two of the hyperthyroid cases

A comparison between $PB^{131}I_{24}$ and C R, $PB^{131}I_{48}$, $Index_{24}$ and $Index_{48}$ is recorded in table I

It has been reported that one not infrequently finds normal $PB^{131}I$ values in hyperthyroid patients with nodular goitre (2, 27) In our series 2 out of 28 hyperthyroid cases with nodular goitre and one out of 52 hyperthyroid patients with diffuse or no goitre had normal $PB^{131}I$ values The reason for the normal $PB^{131}I$ in these cases is not known There is no reason to assume that the examination was affected by iodine medication, which may create a rise in intrathyroid iodine pool and lowered $PB^{131}I$ The proportion between thyroxine and triiodothyronine may be altered so that most of the produced hormone is made up of triiodothyronine (22) Hyperthyroidism with a normal $PB^{131}I$ may represent just this anomaly since triiodothyronine is far more active than thyroxine

The estimation of the conversion ratio in this series gave raised values in most of the euthyroid cases Even with a C R of above 80 % there was a significant overlap In these 3 cases in the euthyroid group who had raised $PB^{131}I_{24}$ the C R was 95 % or more The three hyperthyroid cases with normal $PB^{131}I_{24}$ had C R of 77 % or more

Because of the increased thyroid iodine clearance in iodine deficiency the radio iodine activity in plasma falls quicker than normal leading to a raised value in C R

Eichhorn s modification of the radio iodine plasma test in which one estimates an index from the quotient $PB^{131}I$ at 48 hours and the total activity in plasma at 2 hours is theoretically acceptable Due to iodine clearance in the thyroid being usually significantly higher in hyperthyroidism than in iodine deficiency (1), the total ^{131}I in plasma usually drops sooner in hyperthyroidism than in iodine deficiency (In our series the total ^{131}I in plasma at 2 hours was on an average 2 10 % in euthyroid cases and 1 15 % in hyperthyroid cases) The estimation of this index at 24 and 48 hours in our series, however, gave no more information than could be obtained from $PB^{131}I$

Our investigation has thus shown that $PB^{131}I$ estimation is of great value in the diagnosis of thyroid dysfunction even in an endemic goitre region and that $PB^{131}I$ estimation at 24 hours usually gives as good results as the 48 hour estimation which is more convenient in dealing with out patients In the two hyperthyroid cases with normal $PB^{131}I$ at 24 hours and where $PB^{131}I$ was measured again at 48 hours, the $PB^{131}I$ rose in one case from 0 24 to 0 38 and in the other case from 0 22 to 0 44 Thus when $PB^{131}I_{24}$ is in the range of 0 20—0 35, estimation at 48 hours seems to give somewhat more reliable results

Summary

In iodine-deficiency regions the routine radio-iodine investigations based on ^{131}I-uptake in the thyroid or ^{131}I excretion in the urine are practically valueless if they are not combined with another test aimed at deciding whether a high ^{131}I uptake (or low urinary excretion) is caused by iodine deficiency or hyperthyroidism Most of these tests are time consuming and troublesome

To arrive at a better parameter in an endemic goitre area we have assessed

whereas higher values were found in 71 % of 77 hyperthyroid cases In our series the $PB^{131}I_{24}$ was less than 0 27 % in 38 of 41 "iodine-deficiency" cases (93 %) and more than 0 27 % in 77 of 80 hyperthyroid cases (96 %)

$PB^{131}I$ may be raised in euthyroid people for various reasons, most often due to a small exchangeable iodine pool in the thyroid If the intrathyroidal iodine pool is small, the radio iodine atoms will be diluted into an abnormally *small quantity of stable iodine* and the thyroid will release hormones of a higher radioactivity, despite the fact that the total quantity of the produced hormone is normal (1, 15, 25) This condition has been described after surgical, radiological or medical treatment of hyperthyroidism (20, 25, 27) Such therapy may result in a reduced intrathyroidal iodine pool in euthyroids too (15) The same situation *is obtained in a goitre with marked* degenerative changes (17) and in the case of hyper-active adenoma (12) which by means of its hormone secretion suppresses the hypophyseal TSH-secretion Because of this the rest of the thyroid becomes more or less inactive Raised $PB^{131}I$ *in the case of hypothyroidism* and thyroiditis is generally caused by a small thyroidal iodine pool (1), but it can be assumed that a true rise in iodine-protein secretion occurs in those cases, where PBI is raised (32) It has been shown that even euthyroid patients may have *defects in hormone synthesis* and produce increased quantities of non-butanol extractable iodine which may lead to raised PBI (8, 21) and raised $PB^{131}I$ (8)

In endemic goitre the iodine content per gram of thyroid tissue is only 1/5th of normal (7) and there may be a reduced thyroid iodine pool However, the total iodine content of the enlarged gland is usually within normal limits (14, 15)

Raised $PB^{131}I$ has been described in a euthyroid patient with massive proteinuria (27) In such a case it is possible that the raise may have been due to reduction of the intrathyroid iodine pool due to thyroxine loss in the urine

Raised $PB^{131}I$ has also been described in euthyroid patients with exophthalmos The reason is uncertain, but in these *patients there is often a disturbed iodine* metabolism similar to that in hyperthyroidism (30)

Our experience with the iodine repletion test supports the possibility that iodine deficiency may mask hyperthyroidism (10) Case L F who was *euthyroid and had a raised $PB^{131}I_{48}$* developed a clear hyperthyroidism during the course of the iodine repletion test The patient probably had a hyperactive thyroid even before medication, but due to iodine deficiency was prevented from making sufficient hormone to develop *hyperthyroidism*

The raised $BP^{131}I$ in case L H was probably due to a hyperactive adenoma The scintigram showed a considerable uptake in the adenoma with weak uptake in the remaining thyroid tissue

Cases Å S and S G returned to normal $PB^{131}I$-values after the iodine repletion test of Burrel and Frazer, and the raised $PB^{131}I$ in these cases could have been due to a small exchangeable iodine pool in the thyroid caused by *iodine deficiency*

It has been reported that one not infrequently finds normal $PB^{131}I$ values in hyperthyroid patients with nodular goitre (2, 27) In our series 2 out of 28 hyperthyroid cases with nodular goitre and one out of 52 hyperthyroid patients with diffuse or no goitre had normal $PB^{131}I$ values The reason for the normal $PB^{131}I$ in these cases is not known There is no reason to assume that the examination was affected by iodine medication, which may create a rise in intrathyroid iodine pool and lowered $PB^{131}I$ The proportion between thyroxine and triiodothyronine may be altered so that most of the produced hormone is made up of triiodothyronine (22) Hyperthyroidism with a normal $PB^{131}I$ may represent just this anomaly since triiodothyronine is far more active than thyroxine

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the diagnostic value of estimation of PB^{131}I, conversion ratio and Eichhorn's index

The estimation of PB^{131}I at 24 hours gave a high degree of reliability Only 3 out of 41 euthyroid patients with raised ^{131}I-uptake and lowered ^{131}I excretion showed PB^{131}I-values over 0 27 % of the given dose per litre of plasma, and only 3 out of 80 hyperthyroid cases showed values less than 0 27 %

Estimation of PB^{131}I at 24 hours gave approximately as reliable results as estimation at 48 hours Determination at 48 hours was nevertheless slightly more dependable in the hyperthyroid group Thus when PB^{131}I at 24 hours is in the range of 0 20—0 35 it is advisable to determine PB^{131}I at 48 hours too

The estimation of Eichhorn's index conveyed no more information than could be obtained from PB^{131}I

The conversion ratio was raised in 34 of 41 euthyroid cases

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From the Hospital for Infectious Diseases (Head Justus Strom, M D), Stockholm, Sweden

Acute Alcoholic Hepatitis

A Case with Repeated Attacks

By

JUSTUS STROM

The chronic consumption of alcohol often has a deleterious effect on the liver and its function In particular, its pathogenetic role in cirrhosis has long been known The advance of cirrhosis is thought to be slow and insidious But the course is probably not always so uniform Alcohol may produce acute states of insufficiency in the liver which it is important to recognize particularly from the point of view of differential diagnosis The term acute alcoholic hepatitis has come into use recently It may be of interest to report a case in which no less than three attacks of hepatitis occurred within the space of three years

Case report

A man born on Sept 23 1931 an electrician unmarried looking after his own house He stated that alcohol was no problem There was no case of hepatitis in the vicinity nor known possibility of inoculation

Acute onset on Dec 23 1960 with rigors, 40 C fever muscular pains and nausea Afebrile after 4 days Tired loss of appetite On Jan 16 1961 the skin was observed to be yellow, later the urine had the colour of stout and the faeces were bleached On Jan 26 he was admitted to the Stockholm Hospital for Infectious Diseases with a diagnosis of hepatitis Apart from jaundice he had an enlarged and slightly tender liver Bilirubin 9 6 mg % acute phase protein negative, GOT 165 GPT 132 alkaline phosphatase 33, thymol 1 cholesterol 786 mg % Takata negative Hb 10 5 g % and red blood cells 3 46 million ESR 36 mm/h His condition improved fairly quickly and the values returned to normal (fig 1) During the first two weeks his evening temperature was usually between 37 5 and 38° C An unusual symptom was that he complained of aching and pain over the liver and there was tenderness especially over the gallbladder His white blood cells rose also from 6 900 to 11 900 with a certain shift to the left. At the time of discharge he had increased 6 kg in weight

Apart from the findings shown in the figure it may be mentioned that electrophoresis initially showed low total protein (5 72 g %), low albumin (2 56 g %), and as regards globulins normal quantity of α_1 and α_2, slightly elevated β (0 99 g %), and normal γ The values returned to normal in two weeks Total lipoids were found to be 1000 mg % on Feb 10 Cholecystography showed nothing

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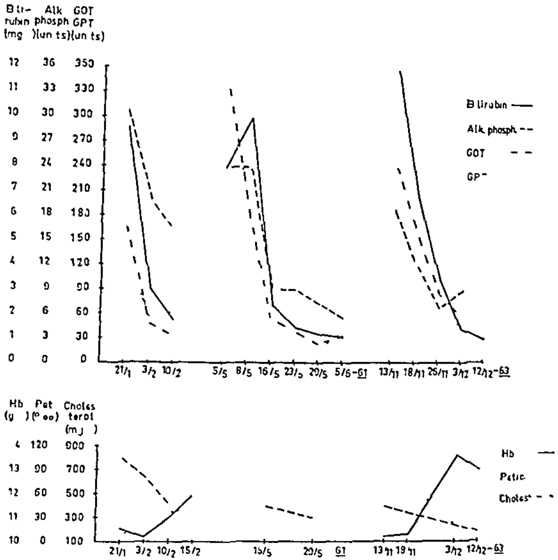


Fig 1 Acute alcoholic hepatitis Three attacks in the same patient

abnormal Diagnosis questionable cholecystitis acuta

Shortly afterwards he again fell ill with loss of appetite, fatigue and jaundice He presented for treatment at Södersjukhuset and was admitted on May 4 1961 He confessed that he drank 1/4 l of spirits every Saturday He was again found to have an enlarged and tender liver (3 fingers breadth below the costal margin) Bilirubin 8 mg % alkaline phosphatase 24 GOT 340 GPT 130 thymol 3 5 Cholesterol 404 mg % Hb 78 % ESR 24 mm/h Electrophoresis showed normal values

Biopsy revealed small fragments of liver parenchyma The material permitted no assessment of the acinous structure as a whole The cells of the liver parenchyma showed heavy fatty infiltration to a considerable extent The periportal connective tissue was markedly increased and contained an increased number of inflammatory cells mostly lymphocytes An increase of yellowish green pigment in the liver cells was also noticeable and in several places typical bile thrombi The condition accordingly revealed a fairly pronounced fatty infiltration with at least incipient cirrhosis accompanied by signs

of bile stasis Pathological report fatty liver with incipient cirrhosis and bile stasis

Cholecystography and cholegraphy showed nothing abnormal

Once again the patient s health was restored fairly quickly and the values returned to normal (fig 1) During the first ten days his evening temperature was between 37 6 and 38° C Diagnosis hepatic cirrhosis

He fell ill for the third time and in the same way in 1963 after apparently having been healthy in the meantime He energetically denied alcoholic abuse but now admitted that he drank 1/2 l of spirits every weekend and in the past four years had also been drinking red wine with every midday and evening meal! The illness started with tiredness and loss of appetite He had lost 3 kg in weight in the past two weeks On admission he was rather thin and taciturn Coarse tremor of the hands No spider naevi Temperature 38 1 C The abdomen was palpable with difficulty A palpable mass was suspected which felt tender under the right costal margin Bilirubin 12 mg % alkaline phosphatase 19 acute phase protein negative GOT 246 GPT 57 thymol 5 Bromsulphthalein showed 18 % retention on Nov 18 and 2 3 % on Dec 3 Cholesterol 385 mg % Hb was low 10 2 g % many reticulocytes (105 ‰) Whites 7 800 Differential cell count normal Serum iron 0 089 mg % Haptoglobin 116 mg % Resistance of erythrocytes normal Diastasuria 256 ESR 36 mm/h Electrophoresis showed normal values apart from elevated β globulin (1 02 g %) Cholecystography nothing abnormal

Biopsy (H Nordenstam) The acinous structure appeared to be largely destroyed The parenchyma was irregularly interlaced with reticular fairly coarse collagenous connective tissue containing inflammatory cell infiltrations and bile ducts Lymphomonocytic and polymorph cells were both present In the intermediate spaces there was parenchymal tissue with residues of acinous structure The majority of parenchymal cells were strongly vacuolized which fat-staining showed to be due to massive fatty infiltration of the parenchymal cells The reticuloendothelium was normal (fig 2)



Fig 2 Liver biopsy van Gieson 1̶0 ×

The course was the same as on previous occasions He regained normal health quickly with all values normal (fig 1) Hb also rose without administration of iron During two weeks or more his evening temperature was between 37 4 and 38 1° C He increased 6 kg in weight in a fortnight

To sum up the patient was a man aged 30 when first taken ill who, up to the age of 33 had three identical attacks of acute hepatic insufficiency He is undoubtedly an alcoholic and after increasing loss of appetite and weight he developed a condition of moderate icterus around 10 mg % bilirubin low fever and tenderness and pain over the liver region Liver tests revealed moderately elevated transaminases with GOT higher than GPT and heavily elevated alkaline phosphatase During an attack the serum albumin is lowered, the β globulins raised but the γ globulins normal The thymol tests likewise show normal values On each occasion of illness he had anaemia against which he had marked reticulocyte response which spontaneously receded during the 3—4 weeks' period of the attacks On all three occasions the attacks were followed by elevated cholesterol The total lipoids

were examined on one occasion and were likewise high Biopsy was done twice and revealed on one occasion destruction of acinous structure and, on both, incipient cirrhosis and, in particular, heavy fatty infiltration of the liver cells and inflammatory infiltrate within the liver tissue On one occasion numerous bile thrombi were also observed

Discussion

The function of the liver can be damaged in many ways by a number of toxic substances, among which alcohol takes a leading place The pathogenesis is quite certainly very complicated It is generally considered that the effect is produced by a changed metabolism due to the consumption of alcohol and an often serious deficiency of other important nutrients, vitamins etc as a result of the alcoholic's often miserable diet

This is usually followed in due course by a change of structure of the liver The primary effect is probably often a fatty infiltration of the liver cells In some of these fatty livers cirrhosis may develop afterwards

These chronic liver affections have been known for a long time But increasing numbers of observations have been made which indicate that acute hepatic insufficiency may follow on the chronic changes Continued alcoholic abuse may thus lead suddenly to an acute deterioration

Philips and Davidson (5) described cases of acute hepatic insufficiency of the chronic alcoholic Popper and Schaffner (6) speak of nutritional fatty liver with hepatocellular degeneration Beckett et al (1) use the expression acute alcoholic hepatitis, and Green et al (3) consider this term the best and most descriptive of the condition

The characteristic features of acute alcoholic hepatitis are that an alcoholic who has been drinking continuously up to the onset of illness

1 suffers a marked anorexia accompanied by loss of weight, tiredness, nausea and low fever,
2 jaundice with enlarged tender liver, and occasionally enlargement of the spleen,
3 pathological liver tests which, apart from bilirubinaemia (usually not above 10 mg %), include moderately elevated transaminases with, in contradistinction to virus hepatitis, usually higher GOT than GPT, not infrequently high values of alkaline phosphatase and sometimes minor changes of the serum proteins (lowered albumin and increased globulin fractions),
4 sometimes anaemia and leukocytosis with monocytosis,
5 characteristic inflammatory and degenerative changes in chronically damaged liver tissue fatty metamorphosis, cell necrosis with inflammatory cell reaction, hyaline degeneration of the liver cells (Mallory bodies), sometimes proliferation of bile ducts or bile stasis, and in most cases incipient cirrhosis

The present patient fits into this picture in all respects Within the space of three years he had three exactly similar attacks But there is another interesting circumstance which should be considered On each occasion the patient also had

hypercholesterolaemia which receded simultaneously with the other symptoms This suggests that he may have a recrudescent Zieve's syndrome which I recently described in this journal (7) This syndrome arises under the same conditions as alcoholic hepatitis and is characterized by jaundice, hyperlipaemia, hypercholesterolaemia and anaemia The liver tests show similar values, with predominance particularly of the cholestasis (alkaline phosphatase) Pain in the region of the liver is also part of the picture The difference is presumably that in Zieve's syndrome the biopsy specimens reveal only the chronic changes but not acute necrotic and inflammatory processes

Quite manifestly these conditions are closely related The difference is perhaps that Zieve's syndrome consists rather of an acute exacerbation of the disturbed lipoid metabolism, reflected in hypercholesterolaemia and hyperlipaemia with secondary cholestasis and haemolytic anaemia, while alcoholic hepatitis represents an acute toxic injury The pathogenetic basis in both cases however is a fatty liver Several of the reported cases of alcoholic hepatitis have also had lipaemia

Under all circumstances both alcoholic hepatitis and Zieve's syndrome represent states of acute hepatic insufficiency As such they probably have a significant role in the development of cirrhosis in the alcoholic The acute attacks, however, disappear quite quickly even if cases with widespread necroses have been reported which may rapidly pass *into* coma followed by death

It is interesting that Beckett *et al* (2) reported instances of alcoholic hepatitis without jaundice This must be a counterpart to anicteric virus hepatitis It may be noted that Zieve's syndrome also appears to have a milder form in which jaundice is absent. This form may be said to include the cases of "lipogenetic" anaemia associated with alcoholism described by Myerson and Gilbert (4)

Summing up these acute illnesses caused by alcoholic hepatic insufficiency on the basis of chronic liver damage in the form of fatty liver, one finds the following scale of syndromes lipogenic anaemia, lipogenic anaemia and jaundice (Zieve's syndrome), anicteric and icteric alcoholic hepatitis All these acute conditions would undoubtedly promote cirrhosis Why certain alcoholics suffer these acute conditions while others gradually and unnoticeably develop cirrhosis is as little known as why only a minor percentage of serious alcoholics develop cirrhosis at all

Alcoholic hepatitis as well as Zieve's syndrome, however, have a great differential diagnostic significance Both may resemble not only virus hepatitis but obstructive jaundice and acute cholecystitis, and the diagnosis should *often* be cirrhosis

Summary

A case is reported of almost identical attacks of hepatitis on three occasions within a space of three years in a 30-year-old man The complex of symptoms accords with the picture of acute alcoholic hepatitis An account is given of the symptomatology of this recently described liver affection with particular reference to the relationship to Zieve's syndrome The two syndromes must be

closely associated and reflect conditions of acute hepatic insufficiency in alcoholics on the basis of a chronically affected liver From the differential diagnostic aspect the two syndromes are of great interest both for the internist and the surgeon

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Autotransfusions at Repeated Treatments with the Artificial Kidney

By

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The indications for dialysis have been much extended in recent years Earlier, dialysis was used mainly in the treatment of acute renal failure The object is to prevent the clinical manifestations of uraemic intoxication (prophylactic dialysis) In the last few years, cases of chronic renal insufficiency have to an increasing extent been subjected to such treatment, since a good technique of arterio-venous shunt etc has become available The increased frequency of treatments with the artificial kidney has brought with it a greater need of blood for transfusion Most artificial kidneys must be primed with 500 to 1,200 ml of blood at each treatment With dialysers that have a smaller volume priming blood is not necessary (2) Some loss of blood cannot be avoided even with these apparatuses This can accentuate the tendency to anaemia which is often present in uraemic patients

The great demand for blood for transfusion at a clinic where active treatment with the artificial kidney is performed with machines that require priming is not only a strain on the blood bank but also a problem that concerns the recipient — the patient A great number of blood transfusions to one patient increases the risk of undesirable reactions At the suggestion of the authors working in the Renal Clinic we have since the autumn of 1963 carried out trials with autotransfusion during treatments with the artificial kidney

We have chosen the designations autoblood for the blood that is collected from the dialyser and autotransfusion for the introduction of this blood *into* the patient concerned The patients who are given autotransfusions have previously received transfusions of donor blood A varying proportion of the blood is thus of homologous and not of autologous type

An account of our autotransfusion technique will be given here The autoblood was checked for sterility and via

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bility Up to the present we have carried out altogether 262 autotransfusions in 32 patients The only contra-indication to autotransfusion was overt septicaemia

Technique

The Alwall type of artificial kidney, 1952 model, was used it holds about 800 ml and the connecting tubes about 200 ml At all treatments a sigma-pump was used for circulation of blood through the apparatus After completed treatment, one of the tubes of the dialyser was sterilized by washing with a detergent solution The tube was punctured with a wide bore sterile cannula which was connected to a 1,000 ml bottle, using an ordinary *collection set The bottle contained* 200 ml of sterile ACD solution (N I H solution B) Air was introduced through the other tube of the dialyser, passing a sterile cotton filter The air forced the blood present in the machine into the bottle The last portion of blood, which had become slightly frothy, was also collected The blood was carefully mixed with the ACD solution and stored in a blood refrigerator with thermostat (+ 2° C — + 4° C) Samples for testing were also taken with the collection set The blood bottle was marked with the patient's name, and other data

When it was time for this patient to be dialysed again after 1 to 10 days, the autoblood was used If the plasma layer appeared haemolytic or otherwise discoloured, and if macroscopical clots had formed, the blood was discarded After specimens had been taken for blood culture, the blood was transferred to the dialyser by means of an ordinary blood transfusion set with a filter If the amount of collected blood was not sufficient to fill the apparatus, a supplement of 6 % Macrodex® or donor blood was given

Bacteriological studies

A prerequisite of such an autotransfusion is that the blood can be withdrawn and kept sterile Before we started our autotransfusion trials, a number of bottles of blood collected by the above-described technique after dialysis were subjected to bacteriological control Twenty-four such bottles were also stored for 20 days and the blood was then examined bacteriologically No bacterial growth was demonstrated in any of the bottles

Blood-cultures were made by the current technique of this hospital, in 2—3 placental *broth tubes, in a Tarozzi broth tube, and in* a blood agar tube The test tubes were incubated for at least 6 days at 37° C They were examined daily by ocular inspection for the presence of macroscopical growth, and thereafter specimens from suspected broths were smeared with a platinum wire on an ordinary blood plate and on an anaerobe blood plate The plates were incubated at 37° C and inspected during 3 days

In the last 78 blood cultures two tubes were also incubated at 20° C but were *otherwise treated as above*

Cultures were made regularly from the autoblood at the same time as the bottle was used The autotransfusion was thus given before the result of culture was known in the individual case No culture revealed any significant growth

On 23 occasions the cultures yielded growths which by the bacteriologists were classified as secondary infections These bacteria were coagulase negative *Staphylococcus albus* on ten occasions, *Pseudomonas* on three occasions, and miscellaneous bacteria on ten occasions All these bacteria occurred in only one out of five to seven media On another occasion non-classified Gram negative rods also grew in four out of five media

Studies of blood viability

Blood for subsequent autotransfusion was collected after dialysis treatment These uraemic patients had received trans

fusions of blood from several donors in connection with one or several previous dialyses To find out whether there was any difference in viability between blood from uraemic and blood from normal donors, we made comparative determinations of the life span of erythrocytes, mechanical resistance, and adenosine triphosphate (A T P) concentration

The *erythrocyte survival* was studied by the method of Garby and Hjelm (4) which is a modification of Mollison's (7) technique of ^{51}Cr labelling The following exceptions, however, had to be made

Before labelling the red cells were kept in ACD solution in the blood bank for 7 days They derived either from the patient's blood collected from the dialyser after the last dialysis treatment or for comparison from healthy donors of a compatible blood-group

The observation period was shorter than that used by Garby and Hjelm (4) The difference in viability of the blood in the first few days was of interest rather than determination of the correct half life More over the observation period could not be extended beyond 7 days in our uraemic patients as after this time most of them needed dialysis transfusion or other therapeutic measures that would have interfered with the results

As with Garby and Hjelm's technique the radioactivity was measured on haemolyzed packed red cells from which the plasma had been carefully removed and the correlation between radioactivity and haemoglobin concentration ("specific activity") in the sample was determined

Fig 1 shows the survival curve for autoblood which had been stored for 7 days before labelling (curve A) homologous red cells which were also given to uraemic patients (curve B) and for

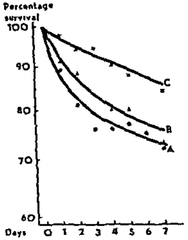


Fig 1 The percentage survival is expressed as ^{51}Cr activity divided by the haemoglobin concentration ("specific activity") per unit volume of haemolysate
Curve A Autoblood labelled and injected into the patient after storage for 7 days Curve B Donor blood labelled and injected into a uraemic patient after storage for 7 days Curve C Autologous blood labelled and re injected immediately The values are taken from Garby & Hjelm's paper

comparison, data from Garby and Hjelm's material (curve C) The survival after 24 hours in this material was 97 5 %, as against 89 % for the autoblood and 91 % for the donor blood

Mechanical resistance was determined by the method described by Shen et al (14) and Schubothe & Fok (12)

To a 50-ml Erlenmeyer flask were added 0 8 ml of packed red cells and 1.2 ml of plasma from the blood to be tested The flask also contained 20 smoothly polished glass beads with a diameter of 4 mm The flask was fixed on a vertical plate rotating at a speed of 40 r p m The rotation was continued for 60 minutes at 22 C. After centrifugation and dilution the haemolysis was measured photometrically at 540 mm using the test plasma as a blank. The obtained value was expressed as a percentage of total haemolysis obtained after dilution with ammonia

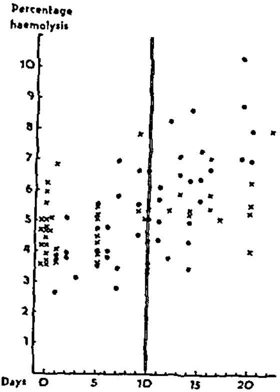


Fig 2 Mechanical resistance as a function of storage time in percentage of total haemolysis × donor blood ● autoblood

Mechanical resistance was measured in autoblood after storage under the above-described standard conditions For comparison, normal ACD blood from conventional donors was also examined The tests were made on blood which had been stored for varying lengths of time after collection from donors and dialysed patients, respectively The results are shown in fig 2 No obvious difference can be read in mechanical resistance between ACD blood and autoblood during the period of actual interest After storage for more than ten days the autoblood erythrocytes seem to be more fragile than those from the donor blood

Adenosine triphosphate was determined on a lyophilic extract of packed red

TABLE I Differences in the concentrations before and after dialysis for bilirubin and haptoglobin

Dialysis treatment	With autoblood	Without autoblood
Total bilirubin in serum mg/100 ml	+0 21 (n=73) (range −0 5–+3 1)	+0 30 (n=26) (range −0 2–+2 0)
Haptoglobin in serum mg/100 ml	−35 (n=67) (range −183–+149)	−48 (n=23) (range −141–+13)

cells, by the enzymatic method of Adam (1), using a commercial reagent (Boehringer)

The results were expressed in mg of A T P per 100 ml of blood with a hypothetical haematocrit of 40 % In conventional ACD blood the A T P concentration was 20 0 ± 0 8 (S E of mean) after storage for 0 to 7 days The corresponding figures for autoblood were 16 6 ± 1 0 The difference is significant on the 99 % level

Serum-bilirubin was determined by Nosslin's (10) modification of Jendrassik and Grof's method *Serum-haptoglobin* was determined by Jayle's method as modified by Nyman (11) Samples were taken before and after dialysis in the cases concerned which were chosen at random among the patients The differences between pre dialysis and post dialysis bilirubin and haptoglobin concentrations in serum will be seen from table I As regards the bilirubin only the values that were maximally 1 2 mg per 100 ml before dialysis are recorded The haptoglobin values include only

those which neither initially nor finally were on the zero-level No other selection of the material was made

Clinical observation

Priming with autoblood was carried out at 262 dialyses of 32 patients The highest number of autotransfusions at dialysis of one patient was 47 At the first 49 dialyses with autotransfusion no further blood was given during the first three hours, so that any reaction could be observed During the final phase of dialysis donor blood usually packed red cells was transfused to counteract the anaemia which develops in patients with long lasting uraemia

Autotransfusion has not increased the frequency of slight rigors which occasionally occur during dialysis treatment No rise in the incidence of bleeding and thrombocytopenia has been noted since the autotransfusion technique was introduced

One patient developed shock within 1 hour of the beginning of dialysis This patient had tubular nephritis due to a septic intra abdominal process which could have caused or played a part in causing the shock There was no evidence of increased haemolysis

Comments

Autotransfusion has in recent years been reported by for instance Walther (16) and Vermeulen Cranch (15) Among the advantages that have been emphasized are that the method assures compatibility and that it can be used in patients who will not accept other persons' blood on religious grounds Shaldon et al (13) have reported that, in using the artificial kidney, they keep the whole dialysing unit which can be used up to 8 or 10 times

Several of the artificial kidneys now in use are, however, of such a design that they cannot be kept and used again without special arrangements The autotransfusion technique devised by us is suitable for these machines

Blood from healthy donors is contaminated with bacteria in 2—5 % cases Such blood has, however been found to possess the property of autosterilization (8) Whether this property would also be characteristic of uraemic blood is not known

The investigated series includes many infected patients and many who received antibiotic therapy The possibility that these factors influenced the bacteriological results cannot be excluded However a significant growth of bacteria was not obtained in any of our cases

As was mentioned earlier the patient has at the termination of dialysis a population of autologous and homologous red cells, which would reasonably influence the survival curve The (insignificant) difference between curves A and B in fig 1 can probably be explained by considerable numbers of up to 20 days older homologous red cells in the autoblood When the first excessive haemolysis has receded curves A and B, fig 1 tend to become parallel to the normal survival curve C from Garby and Hjelm's (4) material This may indicate that the survival times are similar for the rest of the erythrocytes in the examined groups and thus that these are

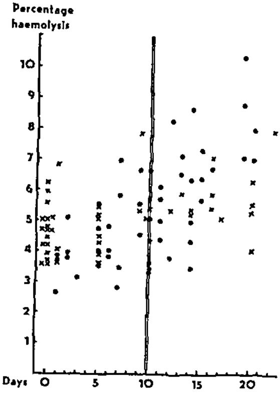


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After dialysis, the blood is forced out of the dialysing apparatus into a sterile glass bottle with ACD solution by sterile air The blood unit ("autoblood") is stored at + 4° C to be used for priming the machine at the next dialysis Donor blood is given to the patient during dialysis if further priming is necessary, or if the patient is anaemic, bleeding, or in shock The method has reduced the demand for blood by 50 per cent This technique has up to now been used 262 times The highest number of autotransfusions at dialysis of one patient was 47

Bacteriological studies have not shown significant bacterial growth in any case Studies of blood vitality *in vivo* and *in vitro* have shown only small differences between donor blood and autoblood

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comparable Yet, the possibility cannot be excluded that renal insufficiency will lead to a diminished excretion of ^{51}Cr, which causes a lower gradient for chromium ions between the two sides of the red-cell membrane and also a higher ^{51}Cr-concentration in "trapped plasma"

The post-transfusion survival of the blood from the previous dialysis (the autoblood), as determined by ^{51}Cr-studies, does not differ from the values reported by Mollison (8) for blood which had been stored for 14 days in the blood bank Thus, as far as the survival of red cells is concerned, our studies have provided no argument against the replacement of ordinary transfusion blood by autoblood

In-vitro studies can also yield information on the condition of the blood Ferber and Fischer (3), for instance, used determination of mechanical resistance in studies of blood-preserving methods A good correlation has also been found (9) between survival and concentration of nucleotides, notably A T P, at the time of transfusion Tests by these two methods showed that there is no considerable difference between autoblood and blood from healthy donors

The rise in serum bilirubin, shown in table I, refers to total bilirubin As the conjugated bilirubin is dialysable, the slight tendency towards an increase signifies a minimum value for the increase of total serum-bilirubin

The bilirubin level has been found to rise within 60 minutes of infusion of non-viable red cells (5) This rise is roughly proportional to the magnitude of the haemoglobin destruction (6) From our results it can therefore be concluded that red cells in autotransfusion blood have a viability that is similar to that of red cells in ordinary donor blood

If the level of the intravascular haptoglobin haemoglobin complex is raised, the method used will result in too low values (11)

Because of the combination of increased bilirubin and decreased haptoglobin, some intravascular haemolysis is probable As donor blood was not infrequently given during the final phase of our dialyses that were started with autoblood, it is impossible to say whether autoblood produces less haemolysis than does ordinary donor blood It can be stated, however, that haemolysis does not occur to a higher degree with autoblood

Since the autotransfusion technique was introduced at this artificial kidney centre, the need of donor blood has been greatly reduced The total number of blood transfusions given at the centre in 1963 was 1460 In the first half of 1964 there has been a 50 % reduction in the required amount of donor blood, despite an increased frequency of treatments

Summary

Frequent dialyses of patients with the artificial kidney, which requires priming with blood, create demands for blood which may be difficult to satisfy The problem can be solved by using dialysers which do not need priming or by re-using the blood from the dialyser at the next treatment We have investigated the latter alternative

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The Institute of Bacteriology (Head R Grubb M D), University of Lund Sweden

Precipitins Against an Antigen Extract of Aspergillus Fumigatus in Patients with Aspergillosis or Other Pulmonary Diseases

By

ROLF BRONNESTAM and TORGNY HALLBERG

Recent observations have increased the interest of clinicians and bacteriologists in pulmonary diseases due to infection with Aspergillus fumigatus Since the atmosphere is contaminated with spores of this fungus it is not always possible forthwith to assess the significance of growth of such a fungus on culture It is therefore desirable to check positive cultures with antibody studies

The first to study antigen structures of the Aspergillus species was Matsumoto (6) who used precipitation and complement fixation reactions Later researchers have worked with both the complement fixation technique (12) collodion agglutination (14) and precipitation in gel (1 3 5 7 8 10) Experience has proved the gel diffusion technique to be particularly useful in such investigations

The purpose of the present investigation in which the gel diffusion technique was used was to study sera from normals from persons infected with Aspergillus from persons with bronchial asthma and from persons with other pulmonary diseases, for antibodies against an antigen prepared from A fumigatus

The investigation also included examination of the ability of the demonstrated antibodies to cross react with antigens of other fungi including Hormodendrum and Candida albicans, and comparison of the precipitation lines against Aspergillus antigen of a variety of sera for reactions of identity and non identity

Material and methods

The immuno-diffusion technique of Wadsworth (16) was used with 1 % agar gel in saline The tests were usually carried out at room temperature The wells containing antigen and antisera were as a rule filled on two consecutive days and the experiment was stopped after 4—5 days

ANTIGENS

Aspergillus antigen

The Aspergillus antigen used consisted of a dried preparation of A fumigatus kindly supplied by Dr Pepys University of London

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TABLE I Occurence of Aspergillus precipitins in sera studied

Group of pat	No of pat	Pos	Neg
Normals	34	0	34
Aspergillosis	5	5	0
Bronchial asthma	15	4	11
Other pulm diseases	24	15	9
Total	78	24	54

25 mg of this powder was suspended in 1 ml of Coca's solution In the gel diffusion tests this suspension was usually diluted 1 4 in Coca's solution

Other fungus antigens

Commercial allergen extracts of the following *fungi in Coca's solution ('stock solution* conc 1 10' A B Vitrum, Stockholm) were used *Penicillium, Mucor, Botrytis,* Alternaria, and Hormodendrum

An antigen extract of Candida albicans was prepared at our laboratory by a modification of a method described by Davies (2) C albicans was cultured in placental broth for 15 days, during which the suspension was continuously stirred After the suspension had been centrifuged and the centrifugate washed with saline, about a 3 % (v/v) suspension of C albicans was mixed with an equal volume of 1 N trichloracetic acid at + 1° C The mixture was then allowed to stand for 3 hours, during this time it was stirred It was afterwards dialysed for 5 days against saline at + 1° C The content of the dialysis tubes was freeze dried Of the powder obtained, 75 mg was suspended in 0 5 ml of Coca's solution and used as antigen

PATIENTS' SERA

Sera from 4 groups of patients were studied

One group consisted of sera from 34 apparently healthy seamen and pregnant women Blood samples from these persons were sent to the laboratory for routine examination of the Wassermann reaction

The remaining 3 groups consisted of sera from inpatients of the Medical Clinic and the Chest Clinic, Lund University Hospital and from Broby Sanatorium, Broby A few sera were also obtained from other departments The groups were made up of the following sera

Sera from 5 patients with verified infection with Aspergillus

Sera from 15 patients with bronchial asthma including 1 with eosinophilic pulmonary infiltrates

Sera from 24 patients with pulmonary diseases other than bronchial asthma or known infection with Aspergillus This group comprised of selected severe cases of sarcoidosis tuberculosis lung cancer, collagen diseases with pulmonary lesions, suspect infections with fungi, pulmonary changes of unknown origin and 3 cases of eosinophilic pulmonary infiltrates

All the sera were stored at — 20° C Clinical data, which were obtained from the patients' hospital sheets, were not studied until after examination of the sera

TESTS FOR SPECIFICITY

Ten sera with Aspergillus precipitins were studied by gel diffusion technique against antigen preparations of Penicillium, Mucor, *Botrytis, Alternaria, Hormodendrum, and* Candida albicans Sera containing precipitins *against any of these fungi were studied* further for reactions of identity according to Ouchterlony If a reaction of identity was observed when a serum was tested against two different fungus antigens attempts were made to absorb the serum with the antigens in question A serum sample was diluted with an equal volume of fungus antigen suspension and placed at + 37° C for 3 hours and then at + 1° C until studied by the gel diffusion technique Serum diluted with an equal volume of Coca's solution was used as reference

Results

All the 34 sera from healthy persons proved negative in precipitation tests with A fumigatus antigen (table I) In

TABLE II Patients with verified infection with Aspergillus

Diagnosis	History	Culture[1]		Steroid therapy	No of precipitation lines
		A fumigatus	C albicans		
Aspergilloma	6 years	3/3	3/3	+	10
Aspergilloma	6 years	0/2	0/2	–	6
Aspergilloma	1 year	1/2	2/2	–	12
Aspergilloma	1 year	3/3	2/3	–	5
Aspergillus pneumonia	3 weeks	7/11	0/11	+	2

[1] No of pos cultures/no of cultures done

TABLE III Patients with bronchial asthma and other pulmonary diseases other than aspergillosis

	No	Culture[1]		Steroid therapy
		A fumigatus	C. albicans	
Patients with precipitins against A fumigatus	19	2/9	4/9	10
Patients without precipitins against A fumigatus	20	2/5	2/5	6

[1] No of patients with pos culture/no of patients investigated by culture

all 5 patients with aspergillosis the results of the precipitation tests with Aspergillus antigen were positive In some of the patients with bronchial asthma or other lung diseases the results were positive while in others they were negative

Further data on the patients with a firm diagnosis of infection with Aspergillus are given in table II

The first 4 patients had a long history and roentgenographic pulmonary changes characteristic of aspergilloma The number of precipitation lines in these 4 cases was larger than in the fifth in this group In this fifth case the history was shorter The patient had severe liver damage and he had received extensive steroid therapy, during which he had developed pneumonia Culture of sputum repeatedly gave growth of A fumigatus

The 15 patients with bronchial asthma and the 24 patients with pulmonary diseases other than asthma or aspergillosis are accounted for in table III

Four of 15 patients with bronchial asthma had antibodies against A fumigatus In these 4 patients the duration and severity of the disease did not differ from that in the remaining 11 patients One patient with bronchial asthma and eosinophilic pulmonary infiltrates had no antibodies against A. fumigatus Nine patients with asthma had received heavy

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In table IV the positive sera are classified according to the number of precipitation lines they produced

Nineteen of the 24 sera had given 1 or 2 precipitation lines in the gel diffusion tests One serum gave 3 lines The remaining 4 sera gave 5, 6, 10 and 12 precipitation lines respectively, in the gel diffusion tests by Wadsworth s method These 4 sera emanated from patients with aspergillomas

Of the 10 patients with Aspergillus precipitins whose sera were tested against other fungus antigens 6 had precipitins against Hormodendrum and 6 against Candida albicans No antibodies could be demonstrated against the other fungus antigens studied In 4 cases the precipitation lines against the Aspergillus antigen gave reactions of identity with those formed against the Hormodendrum antigen The former as well as the latter lines could be removed by absorption with the Aspergillus antigen but only the latter by absorption with the Hormodendrum antigen In one case of the six studied the precipitate against the Aspergillus antigen gave a reaction of partial identity with a precipitation line produced against the Candida antigen The precipitation line formed by the Candida antigen was so weak that it failed to appear even when the serum used was only slightly diluted Therefore no absorption tests could be done with this serum

Seven sera with precipitins against the Aspergillus antigen were compared with one another regarding reactions of identity or non identity between the precipitation lines Five of these 7 sera gave 1 2 lines the remaining 2 sera



Fig 2 Upper left basin Candida antigen Upper right basin Aspergillus antigen Lower basin Serum from a patient with Candida infection Two precipitation lines can be shown against the Aspergillus antigen Against the Candida antigen can be shown 1 precipitation line which gives a reaction of partial identity with one of the precipitation lines against the Aspergillus antigen

gave more than 2 lines One of the precipitation lines gave a reaction of identity between all 7 sera Between the 2 sera with more than 2 precipitation lines a further 1—2 reactions of identity were shown

Discussion

Several investigators have used the gel diffusion technique for demonstrating antibodies against A fumigatus in sera from patients with Aspergillus infection (1 3 5 8) The number of precipitation lines found has ranged from 0—6 (3) to 5—13 (1 5) In our 4 cases of aspergilloma 5—12 precipitation lines formed This relatively large number of lines suggests extensive immunisation against A fumigatus as does the long histories of these patients This assumption is supported by the smallness of the number of precipitation lines and the short

TABLE IV. Distribution of sera according to the number of precipitation lines produced against A. *fumigatus* antigen

Group of pat.	No. of pos. sera	No. of precipitation lines 1—2	3—4	5—12
Aspergillosis	5	1	0	4
Bronchial asthma	4	3	1	0
Other pulm. diseases	15	15	0	0
Total	24	19	1	4

steroid therapy. Three of these had precipitins against A. fumigatus.

Fifteen of 24 patients with pulmonary diseases other than aspergillosis or bronchial asthma had precipitins against A. fumigatus. No noteworthy difference was found between the distribution of the diagnoses among the patients with and without precipitins in this group except in respect of the 3 patients with eosinophilic pulmonary infiltrate, all of whom had precipitins against A. fumigatus. Of the 15 patients with precipitins against A. fumigatus, 7 had received steroid therapy for one month or more within the last 12 months before collection of blood samples studied, while none of the 9 patients without precipitins had received such treatment for more than 1—2 weeks. Sputum or lung punctate was cultured in 9 and 5 cases respectively. The results of culture of these samples for growth of A. fumigatus or Candida albicans are given in table III.

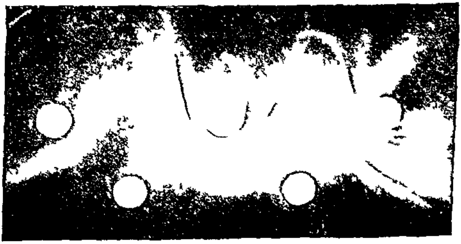


Fig. 1. Upper left basin: Serum from a patient with Candida infection. Upper central and right basins: Serum from a patient with aspergilloma. Lower basin: *Aspergillus antigen*. At least 12 precipitation lines can be shown for the serum from the patient with aspergilloma. At most 2 precipitation lines can be shown for the serum from the patient with Candida infection.

our 4 patients with aspergillomas the number of precipitation lines produced against the Aspergillus antigen was larger than in the rest of the material On the other hand, the number of precipitation lines in the patient with Aspergillus pneumonia was not larger than that in the positive patients with bronchial asthma and other pulmonary diseases In the system used by us demonstration of 5 precipitation lines or more argues strongly for the existence of aspergilloma The occurrence of at most few precipitation lines appears to argue against aspergilloma but does not exclude other forms of pulmonary infection with Aspergillus

It has been shown that A fumigatus and other species of Aspergillus possess some antigens in common (1 5, 6 10) Penicillium (1 4 7), Cladosporium (7 8) and Mucor (1) also possess antigens cross reacting with A fumigatus No antigen cross reacting with A fumigatus has however been found in Sporotrichon schenckii (11) Histoplasma capsulatum (9 11) Blastomyces dermatitidis (9) Bl brasiliensis (11), Pullularia pullulans (11) Alternaria (7), Trichophyton rubrum (1) Geotrichum candidum (1) Cryptococcus neoformans (4 9) or Candida (1 4 7 9) Our specificity tests showed cross reactions between the antigen of A fumigatus and that of Hormodendrum respectively Candida albicans

Some of the demonstrated precipitation lines against the Aspergillus antigen might therefore be the result of infection with some other fungus containing antigen cross reacting with A fumigatus We think it probable that the individual precipitation lines demonstrated in patients with bronchial asthma and other pulmonary diseases except aspergillosis might sometimes be due to such infection, for instance candidiasis

Conclusions and summary

Sera from 34 apparently healthy persons, from 5 persons infected with Aspergillus from 15 persons with bronchial asthma and from 24 persons with various lung diseases were studied by the gel diffusion technique for their reaction with antigen extracts of A. fumigatus and of other fungi

Antibodies reacting with antigen of A fumigatus occur in patients with aspergillosis and, though to less extent, in patients with other pulmonary diseases, but not in healthy persons

The gel diffusion technique may be of value in the diagnosis of pulmonary infections with Aspergillus Demonstration of several precipitation lines argues strongly for aspergilloma At most few lines may occur in other forms of infections with Aspergillus and in other pulmonary diseases

Several fungi pathogenic for human beings have antigens that cross react with antigens of A fumigatus Infections with fungi other than Aspergillus fumigatus may therefore occasionally stimulate formation of antibodies demonstrable also by antigens of A fumigatus

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history in the patient with Aspergillus pneumonia

Pepys et al (7) have shown that A fumigatus occurs more often in the sputum from asthmatics than from normals Pepys et al (7, 8) also demonstrated antibodies against A fumigatus in sera from asthmatics According to Pepys et al (7) in persons allergic to A fumigatus such antibodies may contribute to the production of Arthus-like phenomena and to eosinophilic pulmonary infiltrates In the present material the frequency of precipitins against A fumigatus in the asthmatics was higher than in the normals But it is difficult to appraise the significance of these antibodies for the patients' disease, because the present investigation did not include systematic culture of fungi or examinations of the patients for allergy to A fumigatus antigen

Neither Drouhet et al (3) nor Pepys et al (8) were able to demonstrate circulating antibodies against A fumigatus in patients with pulmonary diseases such as tuberculosis, sarcoidosis and lung cancer On the other hand, Gèrnez-Rieux et al (5), who used the Ouchterlony technique, demonstrated 1 precipitation line produced against an antigen of A fumigatus in 55 of 179 sera from patients with various pulmonary diseases Pepys et al (8) as well as Tomšíková et al (14) found circulating antibodies against A fumigatus in patients with farmer's lung Of our 24 patients with lung diseases other than aspergillosis and bronchial asthma we found antibodies against A fumigatus in as many as 15, which is a much higher proportion than that reported by other authors These differences can probably be ascribed in the main to differences in the material of patients Our patients comprised selected cases with grave pulmonary diseases In the discussion of the significance of the Aspergillus precipitins in the development of eosinophilic pulmonary infiltrates it was interesting to note that all 3 patients with eosinophilic pulmonary infiltrates in this group had antibodies against A fumigatus No antibodies could, however, be demonstrated in the patient with asthma and eosinophilic pulmonary infiltrates

In the group of patients with pulmonary diseases other than aspergillosis or bronchial asthma a certain degree of correlation was observed between the occurrence of precipitins against A fumigatus and steroid therapy

Sidransky et al (13) have shown experimentally the importance of corticosteroid therapy in the causation of infection with Aspergillus In human beings also steroid therapy implies a greater risk of infections with fungi (15) In our material it is possible that the patients with the most severe pulmonary diseases had received steroid therapy to a greater extent than the rest of the patients It is possible that these patients irrespective of the steroid therapy had an increased susceptibility to fungus infections We therefore do not think it is possible to decide if there is, in this material, any connection between steroid therapy and precipitins against A fumigatus

Drouhet et al (3) and Gèrnez Rieux et al (5) have shown that the gel precipitation reaction is useful in the diagnosis of pulmonary aspergillosis In

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Severe Methanol Intoxication

By

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Haemodialysis or peritoneal dialysis has since 1960 been used in a few cases of methanol poisoning in man for the purpose of hastening the elimination of methanol This form of therapy has been recommended as a supplement to the current treatment with ethyl alcohol and alkali (2 17 49 51 54)

In 1961 4 patients with methanol poisoning were transferred to this clinic, all had severe acidosis and were stuporous or comatose at or shortly after admission to a previous hospital They were treated by haemodialysis (in the Addendum are mentioned 2 patients who were later treated by haemodialysis) and with ethyl alcohol and sodium bicarbonate The elimination of methanol and its metabolite formic acid was checked by analysis of serum urine and dialysing fluid Three of the 4 original patients needed artificial respiration These 3 died in the acute phase of the disease the fourth patient recovered but died from another disease 1 1/2 years later Autopsy was performed in all four cases

Submitted for publication July 31 1964

Methods

The dialyses were carried out with Alwall s (3 4) type of artificial kidney The blood flowed through the machine at a rate of 150—300 ml/min The dialysing fluid contained 120 mEq of sodium 4 9—5 8 mEq of potassium 2 8 mEq of calcium 0 8 mEq of magnesium 100 mEq of chloride 35 mEq of bicarbonate and 10 g of glucose per litre It was aerated continously with about 5 litres of carbogen (95 % oxygen plus 5 % carbon dioxide) per minute

The concentration of methanol in serum urine and dialysing fluid was determined by Feldstein and Klendshoj s micro-diffusion method (16) In all the samples the methanol concentration was determined in the acute phase and was also checked by subsequent analyses An improved modification of the method has recently been published by Hindberg and Wieth (25) and was used in the cases mentioned in the Addendum

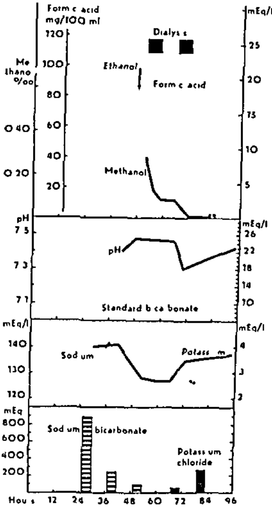


Fig 1 Case 1 Serum levels of methanol formic acid sodium and potassium variations in pH and standard bicarbonate in capillary blood administered amounts of sodium bicarbonate and potassium chloride per 12 hours The arrow *indicates the time at which administration of* ethanol was started The square indicates the duration of dialysis

All the serum and urine samples were also *tested for formaldehyde by Feldstein and* Klendshoj's technique (16) The content of formic acid was determined by the method described by Bastrup (8) Standard bicarbonate, pH and pCO_2 in capillary blood were determined by Sigaard Andersen s et al micro technique (50)

The patients were among 30 cases of methanol poisoning that occurred in 1961 in South Sweden A number of bottles containing 100 % *methanol for* technical use were sold as ethyl alcohol A preliminary report of the four cases *treated by us has been published (32)*

The times given in the report refer to the number of hours after the first intake of methanol

Case reports

Case 1 A 49 year old stevedore who had earlier been in good health and, according to information, was not an alcohol addict At a drinking party he consumed an unknown amount of methanol After 15 hours he experienced failing vision After 24 hours he had abdominal pain, vomiting, giddiness, disturbances of hearing and deep respiration *He was admitted to hospital after 28 hours* with clouding of consciousness and motor restlessness The pupils were dilated and no vision could be demonstrated Standard bicarbonate was 8 mEq/l Soon after admission the degree of wakefulness diminished further and he passed into coma after 29 hours Infusion of sodium bicarbonate was started and continued up to the first dialysis treatment. Because of falling blood pressure, down to 70 mm Hg, treatment with metaraminol (Aramine®) was instituted Tracheotomy was performed after 36 hours

On admission to this clinic after 48 hours *he was in coma, cutaneous oedema* and papillary oedema were noted The eyelid closure reflex and weak pupillary reflexes were present but reaction to *pain was absent* The WBC was 12,800/mm³ and he had neutrophilia Tests with Albustix® and Clinistix® showed proteinuria and glycosuria and the sediment contained large amounts of red blood cells The urine was acid The ESR was 12 mm in an hour

During infusion of sodium bicarbonate pH was 7 48 standard bicarbonate 19 2 mEq/l and pCO_2 21 5 mm Hg At admission to this clinic administration of ethyl alcohol was also started

After 52 hours a 6 hour dialysis was begun, infusion of ethyl alcohol and sodium bi

TABLE I Effect of first haemodialysis in each patient. In cases 3 and 4 the dialysing fluid was changed in the middle of the treatment

			Serum concentration				Concentration in dialysing fluid (140 l)		Total amounts removed	
			Methanol (‰)		Formic acid (mg/100 ml)					
Case	Weight (kg)	Duration (hrs)	Before dialysis	After dialysis	Before dialysis	After dialysis	Methanol (‰)	Formic acid (mg/100 ml)	Methanol (g)	Formic acid (g)
1	97	6	0 275	0 092	104 7	36 8	0 048	11 9	6 7	16 7
2	70	6	0 277	0 072	68 0	28 8	0 033	10 1	5 5	14 1
3	[illegible]	4	0 860	0 370	37 8	11 9	0 088	2 0	12 3	2 8
		4		0 500			0 040	1 0	5 6	1 4
4	[illegible]	4	0 194	0 078		9 5	0 016	7 3	3 3	10 3
		4		0 013		2 2	0 012	1 5	1 7	2 1

carbonate was continued during the dialysis treatment By that time a total of 1200 mEq of sodium bicarbonate had been infused The potassium concentration in serum which before the dialysis treatment was 2 8 mEq/l did not rise despite a high content of potassium in the dialysing fluid The concentrations of methanol and formic acid are shown in *fig 1 and table I*

Respiratory insufficiency necessitated artificial respiration after 55 hours A second dialysis was begun after 66 hours Despite intravenous administration of 65 *mEq of potassium-chloride* and dialysis against potassium rich fluid the serum potassium level rose from [illegible]7 to only 3 4 mEq l At the end of the second dialysis the concentrations of methanol and formic acid were *very low* The patient's condition was unchanged he was in deep coma and had a tendency to a fall in blood pressure After *infusion* of another [illegible]0 mEq of potassium-chloride intravenously during the next 17 hours serum potassium rose to 3 9 mEq l Urinary output [illegible]00—900 ml per day ceased almost completely after 170 hours Large amounts of metaraminol were given up to 1100 mg/24 hours to maintain the blood pressure above 80 mm Hg The patient died *after 136 hours*

Post mortem examination (4 *hours* after death) The brain which weighed 1 550 g was strikingly soft with pressure grooves on the uncus gyri hippocampi and the cerebellar tonsils The convolutions of the brain were flattened The leptomeninges showed greyish red discolouration around large vessels Basally in the subarachnoidal space there was a moderate amount of blood *In both* cerebral hemispheres were large *haemorrhages* (fig 2) on the right side within *the putamen* pallidum and the internal *capsule and* on the left side mainly in the lateral *part of* the putamen and the surrounding area The *haemorrhages* had broken through into the ventricular *system in* several places and through the torn floor of the third ventricle into the subarachnoid space The left pallidum and *the* thalamus were flattened and deformed *The* preserved parts of the putamen and *the* head of the left caudate nucleus were dark greyish red The rest of the brain substance showed diffuse dirty pale greyish



Fig 2 Case 1 Large haemorrhages in both hemispheres

discolouration No thrombi were seen in the sinuses and the basal arteries The lungs showed marked oedema and the bronchi contained profuse viscid turbid secretion The liver showed diffuse fatty degeneration The kidneys weighed together 450 g their surfaces were smooth and pale and the cut surfaces were pale with indistinct markings in the cortical marrow

Microscopical examination There was a massive necrosis of the brain with distinct degenerative changes of the ganglion cells everywhere in the brain such as simple shrinkage, severe cell degeneration (Nissl's "schwere Zellerkrankung") ischaemic cell degeneration karyolysis and disappearance of the cells Even the glia cells were degenerated with pycnotic nuclei karyorrhexis and karyolysis Nowhere were any signs seen of neuronophagia or satellitosis No fatty granule cells were seen The vessel walls were well preserved without perivascular infiltrates The cerebellum was strikingly changed, with total necrosis of the stratum granulosum, loss of nuclei in Bergmann's glia and most of Purkinje's cells The remaining Purkinje's cells showed all degrees of degeneration In the connective tissue around the optical nerve were polymorphonuclear leucocytes, both discrete and in aggregates Staining of the myelin sheath of the optic nerve showed no degenerative changes The connective tissue round the hypophysis also showed infiltrates of leucocytes Nissl's substance in the ganglion cells of the eyes was not demonstrated (staining according to Einarsson's gallocyanin method)

The proximal renal tubules showed varying degree of necrosis with partial shedding of the epithelial cells and the lumina contained numerous granulated cylinders The glomeruli were unchanged

The liver had undergone generalised fatty degeneration without increase of the periportal connective tissue or of inflammation

Methanol and formaldehyde could not be demonstrated in the blood liver, or kidneys

Case 2 A 65 year old stevedore had been operated on for duodenal ulcer but had otherwise been in good health He was an alcohol addict and took part in the same drinking bout as case 1 He consumed an unknown amount of methanol After 15 hours he had nausea, vomiting and headache and after 19 hours failing eye sight After 30 hours he was admitted to hospital He had deep respiration and slight cyanosis of the lips and ears The pupils were dilated but reacted to light and convergence He was colour blind and had visual acuity of about 0 3 The WBC was 16,500/mm³ Standard bicarbonate in serum 11 mEq/l fell despite administration of sodium bicarbonate orally and by infusion to 8 mEq/l after 42 hours Gradually developing coma and respiratory insufficiency necessitated tracheotomy and respirator treatment

After 48 hours he was transferred to this clinic By then he was in deep coma had loss of reflexes, and feeble spontaneous respiration Cutaneous oedema and papillary oedema were noted Falling blood pressure necessitated treatment with metaraminol, which

dur ng the subsequent course had to be g ven cont nuously The WBC was 7 100/mm³ 85 % be ng neutropl ls Prote nur a and glycosuria were d agnosed w th Albustix® and Cl nistix® the sed ment conta ned a large number of red blood cells The d a stase content n the ur ne was normal Blood pH was 7 45 standard b ca bonate 20 0 mEq/l and pCO_2 25 5 mm Hg after a total of 1500 mEq of sod um b carbonate had been g en (f g 3)

Treatment w th ethyl alcol ol was started af er 48 hours D alys s was begun after 53 l ours Dur ng the f rst few hours of the d alys s tl e amoun s of ethyl alcohol given were only sl ghtly ncreased After 5? hours the concentrat on of ethyl alcohol in the blood was 0 2 ‰ Dur ng the next hour the adm n stered amount was increased to 60 g The level n the blood rose to 1 1 ‰ Dur ng he rema n ng 3 hours of d alys s he was g en 94 g of ethyl alcohol and the concen tra on n the blood rose to 2 0 14 g of e l yl alcoh l were fo nd n tl e dial s ng fl d for tl s 3 hour period A second d al s s was begun after 66 hours No cl n cal m l en ent was no ed The pat ent had se ere l ypopo assaem a the lowest value be ng 1 6 m Eq l and was g en a total of 160 Eq potass um-chloride by n ra enous nl on Ol g ria developed w th n about 60 l rs Tl e pat ent d ed with n 79 hours

Po mo em exam na on 36 hours af er [illegible] The bra n wl h we ghed 1 600 g was sof an l tl e leptomen nges around large [illegible] re l v greyısl red The lateral [illegible] na were da k greyish red [illegible] were pal r The rest of tl e [illegible] was pale and d r greyısh red [illegible] part of tl pons were numerous [illegible] s ze l partly confluent [illegible] no ex en l ng n o the en r e [illegible]

Tl e l ngs were oedematous w h areas of a le a s an l sl owed acu e l ronchitis Tl e l er had un lergone marked general zed fa y legenera on The k lneys we gh ng oge her 300 g were sl gl l pale the ortex of he cu surface was somewhat paler than tl e marrow

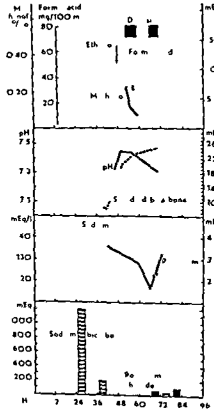


Fig 3 Case 2 Serum levels of me hanol f rm c a d sod um and potass um a a ons n pH and standard b arbona e n cap llary blood adm ms ered am n s of sod um b carbona e and po ass um-chlo de per 12 hours The arrow nd ca es he t me a wh h adm n s ra on of e han l was s arted The squa e nd ca es the lura n of d al s s

M cro op al exam na on The l ra n sho ed rtually the same changes as n case 1 Gangl on and gl a cells exh b ted all stages of degenera on wi hout s gns of react on In the putam na were numerous small per as cular haemorrhages The cerebellum showed necros s of the s ratum granulosum w th loss of Bergmann s glia and some Purk nje s cells

There were no inflammatory nfl rates around the opt cal nerve Nissl s substance n the gangl on cells of the eye could not be demons rated sta n ng accord ng to E nars son s gallocyan n method

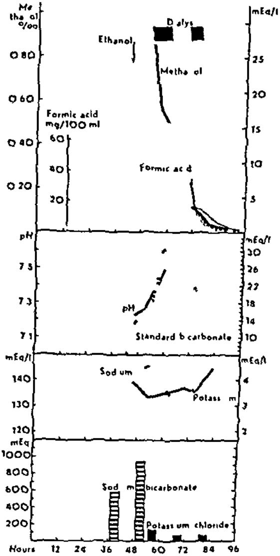


Fig 4 Case 3 Concentration of methanol and formic acid in serum and urine, serum levels of sodium and potassium variations in pH and standard bicarbonate in capillary blood administered amounts of sodium bicarbonate and potassium chloride per 12 hours The thick lines represent the serum levels and the thin lines the urinary levels The arrow indicates the time at which administration of ethyl alcohol was started The square indicates the duration of dialysis

The kidneys showed the same changes as in case 1 Evaluation of the changes was difficult owing to autolysis Methanol 0 27 ‰ was found in the brain but there was none in the blood, liver, and kidneys Formaldehyde was not found in the examined organs

Case 3 A 49 year old waitress who was known to be an alcohol addict drank about 40 g of methanol and 29 hours later another 50 g at least After 40 hours she left work and after 42 hours she was found sitting unconscious at home At hospital, immediately afterwards, deep respiration was noted After 43 hours respiratory standstill occurred and she had a blood pressure fall with impalpable peripheral pulse In response to intubation manual respiration, and infusion of sodium bicarbonate with ethyl alcohol and metaraminol, circulation became satisfactory

At her admission to this clinic after 44 hours tracheotomy was performed and respirator treatment was started By then she was in coma and had slight peripheral cyanosis The pupils were of normal size and reacted to light Reaction to pain and tendon reflexes were absent The WBC was 17,800/mm³ Urine tests with Albustix® and Clinistix® were positive and the sediment contained large numbers of red cells The diastase content in the urine was normal

Blood pH was 7 24, standard bicarbonate 14 1 mEq/l, and pCO_2 30 0 mm Hg after infusion of a total of 600 mEq of sodium bicarbonate (fig 4)

Dialysis treatment was started after 53 hours After 5 hours of dialysis with continued sodium bicarbonate infusion pH was 7 49, standard bicarbonate 31 mEq/l and pCO_2 44 0 mm Hg A total of 1600 mEq sodium bicarbonate by infusion had then been given During dialysis 130 mEq of potassium chloride were infused and serum potassium rose from 3 3 to 3 6 mEq/l At the end of the treatment the pupils were dilated and without reaction to light Slight papillary oedema was noted A second dialysis was begun after 70 hours The patient was still comatose Metaraminol, up to 1100 mg/24 hours was given to maintain the circulation Because of suspected oedema of the brain urea was administered intravenously The depth of coma remained unchanged, however Initially she had polyuria of up to 7 litres of urine per day but oliguria developed within about 100 hours The patient died in circulatory failure within 110 hours

Post mortem examination (4 hours after death) The brain, which weighed 1,600 g was strikingly soft and showed pale dirty greyish red discolouration In the left cerebral hemisphere there was a large haemorrhage which involved the putamen and the surrounding area and had broken into the anterior horn of the lateral ventricle (fig 5) The whole of the right putamen showed a marked dirty greyish red discolouration The ventricular system was filled with blood which via the normal foramina, had entered between the soft membranes on the base of the cerebrum

Pulmonary oedema and tracheobronchitis were present The kidneys showed the same gross and microscopical changes as in case 1

Microscopical examination The histological examination showed the same changes in the brain as in the two previous cases, but in this case there was massive necrosis without reactions of the glia and vessels Nissl s substance in the ganglion cells of the eye could not be demonstrated No methanol or formaldehyde were found in blood liver or kidneys



Fig 5 Case 3 A large haemorrhage in the left hemisphere The ventricular system is filled with blood

Case 4 A 39 year-old house wife who was addicted to ethyl alcohol had for some years had a cough and wheezing respiration She drank about 80 g of methanol After 9 hours she began to have violent vomiting and a few hours later she experienced an ache first in the back and then diffusely all over her body After 36 hours she noticed failing eye-sight On admission to hospital after 44 hours she was blind and had deep respiration She complained of sensory disturbances in the legs Infusion of sodium bicarbonate and ethyl alcohol was started

On arrival to this clinic after 45 hours she had clouding of consciousness She answered when spoken to but her speech was slurred and she was disoriented as to time and place She was not able to distinguish between light and darkness but her pupils reacted to light She had deep respiration and sounds as caused by secretion were heard over both lung fields Despite continuous infusion of sodium bicarbonate pH was 7 09 standard bicarbonate 8 3 mEq/l and pCO_2 17 0 mm Hg Serum potassium was 3 3 mEq/l (fig 6) The urine was acid test with Albustix® was positive and with Clinistix® negative The diastase content in the urine was 512 units (Wohlgemut) The sediment contained large numbers of red and white blood-cells WBC was 17 100/ mm³ 91 % of which were neutrophils The ESR was 102 mm in an hour The patient had a temperature of 39 C Stagnation of bronchial secretion necessitated tracheotomy

Dialysis treatment was started after 50 hours The patient was then comatose and did not react to pain She had satisfactory spontaneous respiration Circulation was maintained with small amounts of metaraminol During dialysis the patient awoke and vision returned At the completion of dialysis within 58 hours she responded adequately when spoken to and could read ordinary newspaper print By then she had received a total of 1 300 mEq of sodium bicarbonate by continuous infusion and pH was 7.58 standard bicarbonate 32 mEq/l and pCO_2 33 mm

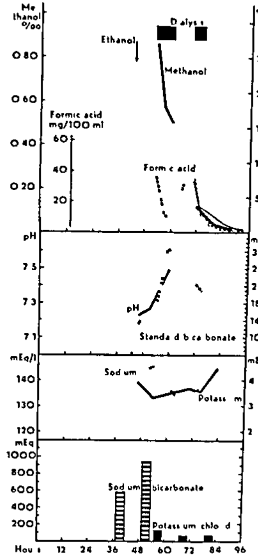


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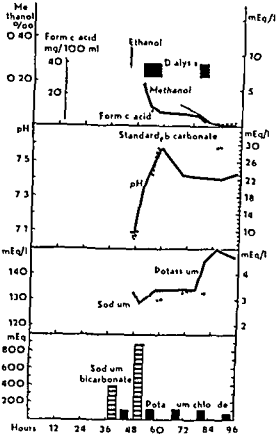


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Hg Despite infusion of a total of 240 mEq of potassium chloride since her arrival at the clinic, serum potassium was unchanged at 3 3 mEq/l Within 76 hours a second dialysis was started An EEG taken after 180 hours showed some slight diffuse theta activity with asymmetrical response to flicker Examination of the eyes after 120 hours showed no abnormalities

The patient never called for a doctor again According to information from her relatives, she caught a cold about 1 1/2 years after the methanol poisoning, had pain in the chest



Fig 7 Case 4 Slit shaped cysts in the lateral parts of both putamina

and the back and high fever Her condition deteriorated and she died after a few days

Post mortem examination In the brain slit shaped cysts were seen in the lateral parts of both putamina (fig 7) There were extensive bronchopneumonic foci notably in the two lower lobes In both lungs were areas of moderate bronchiectasis The spleen exhibited the typical picture of infection The heart was normal, without any signs of right sided hypertrophy Gastric ulcer and diffuse fatty degeneration of the liver were diagnosed

Microscopical examination Round the above-mentioned cysts was a pronounced glia proliferation The cysts were filled with vascularized loose connective tissue and a few macrophages The cerebellum showed in several places small sclerotic areas with loss of Purkinje s cells and stratum granulosum cells as well as proliferation of Bergmann s glia

Discussion

Symptoms and course

In 3 of the 4 cases information is available about the symptoms of intoxication before admission to hospital Two patients fell ill within 9 and 15 hours respectively, of the intake of methanol with nausea, vomiting, and headache One patient had failing eye sight as the first symptom within 15 hours, the other two

experienced impaired vision within 19 and 36 hours, respectively. Deep respiration was noted in 1 patient within 24 hours. In the others this symptom was noted at admission to hospital within 30, 42 and 44 hours, respectively. By then, all had severe metabolic acidosis with standard bicarbonate between 8 and 14.1 mEq/l in 2 cases measured during the course of alkali therapy, which was started within 29, 30, 43, and 44 hours, respectively. Ethyl alcohol was instituted within 48, 48, 43, 44 hours, respectively. No data were obtained proving that the patients had drunk ethyl alcohol at the time of or after the intake of methanol. The first dialysis treatment was started within 52, 53, 53, 50 hours, respectively. All the patients developed hypopotassaemia. All had leucocytosis, neutrophilia, proteinuria and microscopical haematuria as well. Three of the patients had glycosuria. One had a slightly raised level of urinary diastase. Coma occurred within about 29, 36, 42 and 48 hours, respectively. Artificial respiration was required because of pulmonary insufficiency in 3 cases and was started within about 55, 36 and 43 hours, respectively. Oliguria anuria developed in these patients within about 120, 60, 100 hours, respectively, and death occurred within 136, 79 and 110 hours. The fourth patient, who survived her intoxication for 1 1/2 years, died from untreated pneumonia, the development of which was promoted by bronchiectasis.

Post mortem findings

The 3 patients who died as a result of their methanol intoxication had massive necrosis of the brain without reaction of the glia or the vessels. This suggests that the changes were due mainly to autolysis, which started intra vitam. It is sometimes seen after prolonged respirator treatment of deeply unconscious patients with respiratory insufficiency (11, 37). In our cases the respirator was used for 81, 43, and 66 hours, respectively. The massive necrosis of the brain made it difficult to assess the findings that may be considered to result directly from the methanol poisoning, notably the necrosis in the putamina (42). Patients nos. 1 and 3 had massive haemorrhages into the putamina and their surroundings, breaking into the lateral ventricles. The confluent haemorrhages into the pons in case 2 were probably attributable to the increased intracranial pressure. In this case the putamina were strikingly soft and of a greyish red colour as a result of small perivascular haemorrhages, as has also been described by Orthner (42).

The inflammatory changes in the connective tissue around the optical nerve and the hypophysis with a very dense accumulation in some areas of polymorphonuclear leucocytes in case 1, were striking. Whether this inflammation is an effect of the methonal poisoning or not cannot be established. Inflammatory infiltrates in the connective tissue around the hypophysis, though not around the optical nerve, have sometimes been seen in massive necrosis of the brain in unconscious patients given respirator treatment (56).

In patient no. 4, who was not treated with artificial respiration in connection with her intoxication, autopsy showed symmetrical slit-shaped cysts in the

lateral parts of the putamina These changes, as well as the haemorrhages into the putamina in the other cases, correspond to the pattern of injuries which according to Orthner is typical of methanol poisoning

It cannot be established whether the small sclerotic areas in the cerebellum with loss of Purkinje's cells and stratum-granulosum cells and proliferation of Bergmann's glia cells seen in case 4 were residual changes of necrosis in association with methanol poisoning (27) or ethyl-alcohol addiction (38) Such necroses have also been seen in uraemia of various origin (41) and in disturbances of the carbohydrate metabolism — diabetic coma, insulin poisoning (12, 31, 48, 55)

Case 4 is of special interest because it is the first case in which probably residual changes of necrosis in the putamen have been demonstrated in a patient who survived a methanol intoxication

Toxicology

The toxic effects in methanol poisoning are produced in association with the breakdown of the substance and are to some extent correlated to the severity of acidosis that develops The metabolites of methanol — formaldehyde and formic acid — can only partly explain the development of severe acidosis (15, 43, 46, 47) It must also be due to acid radicals that form as a result of secondary metabolic disturbances (47) The formic-acid concentration can to some extent be said to be a measure of the amount of metabolised methanol The concentrations of methanol and its metabolite formic acid in serum and urine of all our 4 patients are shown in table I and fig 1 On their admission to this clinic, within 44—48 hours of the methanol intake, patients nos 1 and 2 had much higher concentrations of formic acid than had patients nos 3 and 4 This means that the first two would have had time to metabolise more methanol than the latter two, which is in agreement with the time interval between the onset of symptoms of severe acidosis and the administration of ethyl alcohol, being in cases 1 and 2 about 20 hours and in cases 3 and 4 probably only a few hours

Varying lengths of time between the intake of methanol and the institution of treatment, as well as uncertain information regarding the consumed amounts of methanol and ethyl alcohol would explain the varying statements concerning the minimum lethal dose of methanol, 25—200 g, found in the current toxicological textbooks (5, 18, 36) Early treatment has prevented death or visual defects even after high consumption of methanol, for instance about 250 and 200 g, respectively, in Roe's cases nos 65 and 77 (46) Untreated methanol poisoning can prove fatal, judging by Roe's series, after intake of about 100 g and lead to transient blindness after 40 g Wieth and Jorgensen (54) described a case in which the patient had drunk about 80 g of methanol The patient died despite adequate bicarbonate treatment started within 18 hours and haemodialysis within 30 hours of the intake In our case 3 about 90 g led to a fatal outcome and in case 4 about 80 g caused transient blindness, coma, and persistent although not clinically manifest brain damage In both these cases the treatment was started first

within about 43 and 44 hours, respectively. Then, one of the patients was in coma and had circulatory failure, and total blindness had developed in the other. On these grounds, the minimum lethal dose of methanol in untreated adults can be set at probably less than 80 g. A risk of visual defects will probably attend the consumption of half this amount, which means an initial serum methanol concentration of about 1 ‰ at a body weight of 70 kg. At a lower serum methanol concentration a severe acidosis as evidence of advanced methanol oxidation may signify that the risk of visual defects and death is imminent. This is illustrated by our four cases in which the concentrations of methanol in serum were low at the beginning of the treatment.

Treatment

It may be considered established that ethyl alcohol inhibits the breakdown of methanol in man and the rhesus monkey (21, 30). According to Bartlett's (7) studies with C^{14} labelled methanol an ethyl alcohol concentration in serum of 0.46 ‰ inhibits the metabolism of methanol to 70 %. Clinical experience indicates that an ethyl-alcohol concentration in the blood of 1 ‰ causes virtually total inhibition of the methanol oxidation (1, 46). The reason why the clinical value of ethyl alcohol treatment in methanol poisoning (46) earlier has been questioned (35 a. o.) would be results of animal experiments with non primates (19, 34) whose methanol metabolism, according to later studies, is not the same as that of man (20).

Our patients were given ethyl alcohol according to the principle devised by Roe (46). The breakdown of methanol seems to have been virtually completely inhibited in cases 1, 2, and 4. In case 3, on the other hand, the methanol concentration fell and the formic acid concentration rose between the two dialysis treatments as will be seen from fig. 4. The patient had, reasonably, not received sufficient compensation for the increased elimination of ethyl alcohol during the dialysis. The figures quoted in the early literature for the rate of breakdown of ethyl alcohol are probably too low. According to later experience (13), the rate of breakdown of ethyl alcohol in a normal person amounts to 10—12 g per hour. The amount of ethyl alcohol removed by dialysis during the treatments had to be replaced by extra administration. There are no earlier experiences with the ethyl alcohol clearance of our dialysing machine and, as will be seen from the case reports, especially case 2, it was difficult to estimate the suitable dose of ethyl-alcohol administration during dialysis treatment. Samples for determination of ethyl alcohol were taken at the first dialysis in case 2. Ninety four grammes of ethyl alcohol were given during the last three hours of dialysis. The patient would break down approximately 30 g in that time. Fourteen grammes were recovered from the dialysing fluid. The remaining 50 g increased the concentration in the blood from 1.1 ‰ to 2.0 ‰. To avoid the difficulties of intravenous administration of ethyl alcohol during dialysis it would be possible to use a dialysing fluid with an ethyl alcohol concentration of 1 ‰.

Treatment with alkali in methanol poisoning attended with acidosis has long been practised (24, 26) Clinical experience and experimental studies in monkeys (9, 10, 44, 54) have shown that visual defects and death cannot always be prevented in methanol poisoning if treatment consists only of correction of acidosis with alkali Probably, the duration of acidosis plays an important role Our findings are not inconsistent with the view that adequate treatment with bicarbonate started at an early stage reduces the risks of toxic effects By intensive alkali treatment the pH of the blood can be corrected quickly A change in intracellular pH is probably corrected much more slowly (14, 45, 53) In our cases 1 and 2, in which alkali was given initially, the patients deteriorated gradually although their extracellular acidosis had been corrected with 1200 and 1500 mEq of sodium bicarbonate, respectively, orally and intravenously The relatively large doses of sodium bicarbonate which must be given over a short time to control an acidosis due to methanol, can produce an increase in the intracranial pressure, convulsions, and signs of pulmonary oedema (9, 46, 52), especially in the presence of renal insufficiency In such cases in particular, dialysis is a safer method for correction of acidosis than is treatment with alkali alone By dialysis treatment, accumulated acid radicals can be eliminated and bicarbonate administered without risk of electrolyte fluid retention

Because of the pronounced hypopotassaemia in our cases we gave large amounts of potassium salts intravenously, and a high potassium dialysing fluid was used Hypopotassaemia in methanol poisoning has earlier been observed (52) Formate formed intracellularly and possibly other anions may in these cases lead to an increased immigration of potassium ions into the cells

Our experiences with haemodialysis are consistent with those of others (6, 17, 49, 54) in showing that the time of elimination of methanol is greatly reduced with this method Similar results have been obtained by peritoneal dialysis (2, 51) Through the natural channels the elimination of methanol and formic acid is slow (fig 6) Table I shows the elimination of methanol and formic acid during the first dialysis in each patient In case 3 the amount eliminated over the first three days in hospital was only 7 g, in a total of 15 litres of urine, whereas 18 g were removed during the first eight hour dialysis The explanation of this, as will also be seen from fig 4, is that the concentrations of methanol and formic acid in the urine are of the same order as those in serum and the rest of the body fluids Forced diuresis which, for instance in barbiturate poisoning, greatly reduces the time of elimination of the poison (39, 40), has therefore a relatively insignificant effect on the elimination of methanol

Post mortem examination of the blood, liver, and kidneys revealed no methanol whereas brain tissue examined chemically in case 2 only, was found to contain 0 27 ‰ methanol The explanation of this finding in the brain is probably interference with the circulation of blood caused by the necrosis of the brain The intracerebral haemorrhages which have been demonstrated in cases of methanol-

induced brain lesions, may have been accentuated by heparinisation during haemodialysis Some form of regional heparinisation is desirable (23, 29, 33 a o) in order as far as possible to cut down the prolongation of the clotting time

On the basis of others (2, 21, 22 28, 46 54 etc) and our own experiences, it seems that the main principles in the treatment of methanol poisoning should be

1) Prompt oral or intravenous administration of ethyl alcohol in order to inhibit the methanol oxidation, about 50 g should be given initially to a 70 kg patient and thereafter about 10—12 g per hour so as to maintain an ethyl alcohol concentration in the blood of about 1 ‰ This treatment should be continued until virtually all methanol has been eliminated
2) Careful clinical and laboratory supervision of the patient including for instance a quick method for determination of methanol and frequent checking of pH and standard bicarbonate In patients with acidosis the serum electrolytes the serum potassium content in particular should be checked
3) Administration of alkali in cases with acidosis Care must be taken in severe acidosis as the amounts of sodium bicarbonate required for correction may involve the risk of oedema of the lungs and brain especially if renal insufficiency is present
4) Haemodialysis or peritoneal dialysis to ensure quick elimination of methanol and accumulated acid radicals The indication for dialysis is strengthened in patients with impaired renal function or acute renal insufficiency as complications of methanol poisoning
5) Tracheotomy and respirator treatment in cases of impending pulmonary insufficiency

Summary

Four cases of methanol poisoning were treated with alkali ethyl alcohol, and the artificial kidney In 3 cases respirator treatment was also required The concentrations of methanol and its metabolite formic acid in blood urine and dialysing fluid were checked

Severe acidosis, failing eye-sight, and cerebral damage were present at the start of the treatment within 29 to 44 hours of the intake of methanol Three patients, one of whom had consumed 90 g died despite correction of the acidosis and elimination of methanol Autopsy in these cases showed massive necrosis of the brain and haemorrhages into the putamina The fourth patient who had consumed 80 g of methanol, survived but died 1 1/2 years later from pneumonia, autopsy showed slit shaped cysts in the lateral parts of the putamina All the patients had considerable hypopotassaemia

By haemodialysis the time of elimination was greatly reduced for methanol as well as for formic acid

The most important procedures in the treatment of methanol poisoning should be careful clinical and laboratory supervision of the patient inhibition of the methanol oxidation with ethyl alcohol and administration of alkali to patients

with acidosis By dialysis treatment the time of elimination of methanol can be greatly reduced and acidosis can be effectively corrected

Addendum

Five patients were later observed in this clinic after consumption of 300—500 ml of spirits intended for technical use These can contain a small percentage of methanol, among other impurities 2 5 g of methanol per 100 ml were demonstrated in the kind of spirit consumed by one of these patients At ingestion of such spirit the amount of methanol, which is inconsiderable in relation to the quantity of ethyl alcohol is usually of no clinical significance

Two of these patients were treated with the artificial kidney

1. S L (male, 32 years of age) had drunk 500 ml of methylated spirit He had abdominal pain and a history of previous pancreatitis During a 6 hour dialysis, 5 g methanol were eliminated with the dialysing fluid The concentration of methanol in serum was reduced from 0 24 to 0 14 ‰
2. K J (male, 27 years of age) had consumed 400 ml of methylated spirit His methanol concentration in serum was inconsiderable whereas the ethyl alcohol concentration was 4 1 ‰ which was not known at the crucial moment in the acute phase He had visual disturbances and urinary bladder paresis but no acidosis Alkali had been given at the admitting hospital

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Medical Department VII (Head E M Blegen, M D) Department of Pathology (Head K Arnesen M D) and The Central Laboratory (Head S L Sveinsson, M D), Ullevål Hospital Oslo Norway

Oxalosis

A Case Report

By

ERIK ENGER, ARNE SERCK HANSSEN and TORGEIR ROKKONES

Oxalosis or primary hyperoxaluria is a rare genetic disorder of glyoxylate metabolism which is clinically characterized by calcium oxalate urolithiasis nephrocalcinosis recurrent urinary infections and finally by disseminated extrarenal calcium oxalate deposits The disease usually appears in early childhood with progression to death in uremia within few years The occurrence of oxalosis in siblings suggests its transmission as an autosomal recessive trait but no deficit in persons assumed to be heterozygotes has been found Up to 1963 the occurrence in 11 families has been recorded (3) An extensive review of the condition has been presented by Wyngaarden and Elder (5)

Biochemically the disorder is characterized by a continuous high urinary oxalate excretion There is no evidence of an increased absorption of dietary oxalate (1) The primary defect is therefore related to a metabolic disorder leading to an excessive biosynthesis of oxalate which is metabolically inert and has to be excreted or deposited (2) Glyoxylate is the only compound which has been clearly defined as an *immediate* oxalate precursor in mammalian systems Frederick et al (3) demonstrated an impaired metabolism of C^{14} labelled glyoxylate to carbon dioxide in patients with primary oxaluria This suggests a defect in the metabolism of glyoxylate to formate and carbon dioxide But an increased glyoxylate pool due to a different cause can not be excluded The rate of the glyoxylate formation and the size of the glyoxylate pool in patients with primary hyperoxaluria remain to be measured We have to conclude that the excessive synthesis of oxalate is secondary to an obscure defect in the metabolism of glyoxylate The renal handling of oxalate in these patients is unknown But the increased urinary excretion of oxalate must in some way be related to an elevated blood level With progressive deterioration of the renal

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function due to recurrent calculi and infection, oxalate is retained in the body with consequently disseminated renal and extrarenal deposits

The present case is reported for the following reasons 1 There has been no previous report of oxalosis in the Scandinavian literature, 2 the patient presented a puzzling clinical picture, 3 some of the pathological findings were unusual and 4 the case emphasizes the importance of urinary oxalate determination in instances of recurrent renal stones in infancy

Case report

The patient was a woman born in 1938 as the youngest of 4 children One sibling died as a baby of congenital heart disease Two siblings have always been in good health and have never experienced symptoms or signs suggestive of renal infection or calculi

There was consanguinity between her parents The father married his first cousin's daughter and thus the patient and her mother were second cousins

From the age of 2 she had recurrent urinary infections Two years later roentgenological examination showed a large concrement in the right renal pelvis and a nephrectomy was performed A soft and large concrement was occupying the renal pelvis There were also several small and hard calculi localized at the renal papillae Histological examination revealed signs of advanced chronic pyelonephritis Postoperatively, the urine was normal by chemical and microscopical examination

Later she developed normally in every respect and did not again experience symptoms and signs of urinary infection or calculi

Aged twenty three years she went through a normal pregnancy with normal urine and blood pressure by frequent controls

During the autumn of 1963 she developed progressive weakness She was admitted on 30th Nov 1963 to the Medical Department VII of Ullevål Hospital as an emergency case. She was in an extremely poor condition The pulse was irregular varying between 45 and 60 per minute The B P was 90/70 mm Hg The respiration was deep and frequent. Blood urea was 380 mg/100 ml and the total CO_2 content of plasma was 6 mEq/l

During the subsequent days the following clinical and biochemical features were noted

Renal insufficiency and an abnormal urine

On admission the serum creatinine concentration was 18 mg/100 ml The creatinine clearance varied between 5 and 10 ml/minute Initially, the daily urine volume was about 1000 ml The urine was slightly acid with a constant specific gravity of 1012 There was a moderate proteinuria varying between 1 and 2 g per day On microscopical examination abundant white blood cells of the Malbin Sternheimer type were noted There were also several granular and waxy casts Urine culture revealed coliform bacteria Roentgenological examination showed a normal sized left kidney, i e without the compensatory hypertrophy which might have been expected There were also a few small shadows suggestive of renal calculi

Cardiovascular involvements

Except for a transitory period shortly after admission daily ECGs showed a complete heart block during the entire hospital course The appearance of the ventricular complexes was steadily changing Initially, the heart rate was about 40 per minute Peroral administration of ephedrinehydrochloride supported by subcutaneous injections of epinephrine kept the heart rate between 50 and 60 per minute A short and intense course of peroral methyl prednisolone was without effect on the conduction disturbance During the final 2 weeks the heart rate remained at about 50 per minute without therapy The blood pressure was normal during the entire course Apart from the rhythm disturbance physical examination of the heart was normal

After one week in hospital she developed a peculiar acrocyanosis of fingers and toes with progression to superficial areas of ischemic

necrosis They were painful and required administration of morphine derivatives

Other laboratory studies

On admission the Hb was 7 3 g/100 ml, and the R B C 3 4 mill /mm³ A blood smear revealed hypochromia and microcytosis The W B C was 7000/mm³ with a normal differential count There was no evidence of hemolysis The bone marrow was normal and repeated L E-cell preparations were negative Except for an initially high serum potassium level the concentration of this ion remained within normal limits The serum sodium and chloride concentrations were within normal limits During the first 3 weeks there was a pronounced hypocalcemia with serum calcium levels varying between 7 and 8 mg/100 ml During the last 2 weeks the serum calcium concentration rose to 10 mg/100 ml The serum phosphate level varied between 7 and 8 mg/100 ml The serum magnesium concentration was 2 0 mg/100 ml The protein content of serum was normal while paper electrophoresis showed a moderate hypoalbuminemia

The clinical course

She responded favourably to the initial emergency treatment Antibiotics were administered to influence the pyelonephritic process During the entire course she had periods with muscular twitchings but tetanic phenomena were not observed Owing to the low serum calcium level 10 ml of a 10 % solution of calcium gluconate was given intravenously once a day the first 3 days after admission During the last part of the hospital course when peritoneal dialysis was performed such injections were given every second day The possibility that the ischemic skin lesions were in some way related to the administration of the sympathomimetic agents was considered After two weeks these were therefore avoided She was given small transfusions with packed red cells sufficient to keep the hemoglobin content at about 10 g/100 ml

After 3 weeks in hospital there was a further deterioration of her clinical condition At this time oliguria developed with a constant rise of serum creatinine and urea levels Because of her age and the uncertain diagnosis peritoneal dialysis was started Initially she improved satisfactorily But 37 days after admission the condition suddenly deteriorated She experienced intense pain in the upper part of the abdomen associated with vomiting and signs of peritoneal involvement The peritoneal fluid became hemorrhagic and a perforation was suspected Few hours later she developed a circulatory collapse and died

Post mortem examination

There was incipient gangrene of all toes and considerable edema of both lower extremities

Each pleural cavity contained 200 ml hemorrhagic fluid The pericardial cavity was normal The heart weighed 350 g The valves the chambers and the coronary arteries were normal

The aorta showed insignificant, atheromatous plaques surrounding the orifices of its branches From the lower half of the femoral artery there were intimal changes with areas of a depressed scarlike appearance due to a thinning out of the media These changes continued as far as the arteries were opened No thrombi were found

The peritoneal cavity contained 500 ml hemorrhagic fluid There was no sign of traumatic injury

The pancreas was enlarged with a weight of 200 g It was edematous with a nearly complete hemorrhagic necrosis There was no sign of fat necrosis There was no thrombosis of the splenic vein or of the central parts of the pancreatic arteries

The liver the spleen, the gall bladder and the bile ducts were normal

The left kidney showed signs of advanced pyelonephritis The renal pelvis was thickened and dilated The ureter was also thickened but there were no stones in the renal pelvis ureter of bladder

Histological examination

The kidney There were signs of an advanced chronic pyelonephritis Masses of birefringent crystals were filling the lumen of most of the

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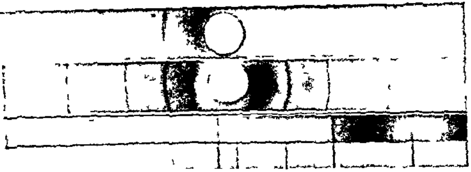


Fig 5 a X ray diffraction pattern (Debye Scherren camera)
1 Crystals from renal tissue
2 Calcium oxalate monohydrate
b X ray diffraction pattern (Guinier camera)
1 Crystals from myocardium
2 Calcium oxalate monohydrate

morphology as in the kidney The crystals were mainly found in the interstitial tissue usually associated with considerable fibrosis and some degree of foreign body reaction (fig 3) Occasionally the crystals were apparently located inside the myocardial fibres with a slight tissue reaction only Probably these were the most recent deposits *Crystals were also found in the media of small* arteries and arterioles

The pancreas Sections from all parts showed an extensive hemorrhagic necrosis with thrombosis in several small arteries and veins *There were some areas of fat necrosis* As a prominent feature there were abundant deposits of crystals in the media of arteries and arterioles associated with a moderate reaction from the vessel wall and the presence of mural thrombi

Tissue from the right 1st toe There were patchy areas of ischemic necrosis and extensive deposits of crystals in the media of arteries and arterioles Many of these were occluded by thrombi Others contained small mural thrombi at the site where the crystals came close to the intima (fig 4)

Brain lung and liver No crystals or other changes were found in the vessels or the tissue of the brain and the liver There were a few crystals in the arteries and arterioles of the lungs Otherwise the picture was that of uremic lungs

Examination of the crystals

The morphology of the crystals showed features which suggested calcium oxalate They did not stain with hematoxylin and eosin and were also left unstained by the van Kossa s *staining technique* By the X ray diffraction technique the crystals were identified as calcium oxalate monohydrate (fig 5a and b)

Comments

The possibility of oxalosis was not considered during life Owing to the past history and the present demonstration of small calculi in the remaining kidney various other metabolic causes of nephrocalcinosis were considered However primary hyperparathyroidism renal tubular acidosis and cystinuria were all thought unlikely as explanations of the clinical picture As a conclusion, therefore, the condition was interpreted as the terminal phase of chronic pyelonephritis associated with unusual cardiovascular features

Presumably the acute hemorrhagic necrosis of the pancreas was the imme

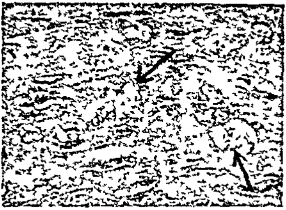


Fig 1 Medullary part of the kidney with calcium oxalate crystals mainly in dilated tubules indicated by arrows Diffuse interstitial inflammatory reaction Half crossed polarizing filters Hematoxylin and eosin × 50

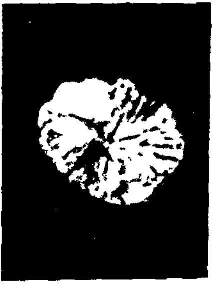


Fig 2 Calcium oxalate crystal from myocardium Crossed polarizing filters × 1430



Fig 3 Myocardium with multiple calcium oxalate crystals fibrosis and some foreign body reaction Half crossed polarizing filters H & E × 300



Fig 4 Small digital artery from the toe with multiple calcium oxalate crystals in the media Arrows indicate points of attachment of a small mural thrombus Half crossed polarizing filters H & E × 150

renal tubules, many of which were markedly dilated The crystals were found both in the proximal, distal and collecting tubules There was also large amounts of crystals apparently in the interstitial tissue (fig 1) Morphologically, the crystals appeared similar to the calcium oxalate crystals seen in cases of ethylenglycol poisoning and in cases of primary hyperoxaluria (fig 2) Sections from the kidney removed 22 years earlier were re-examined These showed signs of severe chronic pyelonephritis with occasional small crystals in the tubules These, however were only observed by polarized light

The myocardium Sections from various parts of the interventricular septum, from His Tawara's bundle and the posterolateral part of the left ventricle showed extensive deposition of birefringent crystals with the same

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Continuous Intragastric pH Determination

I. pH of the Gastric Juice Determined in Situ and Following Aspiration

By

MARIE KRISTENSEN

Investigation of gastric acidity is one of the most important gastric tests. Ewald's test meal has been replaced by aspiration of fasting secretion, in some cases combined with maximum histamine stimulation (5, 14). Determination by glass electrode gives, in contrast to the titration methods, a direct determination of the pH. This is decisive in ascertaining whether the proteolytic enzymes, primarily pepsin, have a possibility of functioning. Calculation of the pH on the basis of the number of titrated acid equivalents is unreliable as the result is influenced by the uncertainty of the titration as well as by the ionic strength of the solution and the buffer systems present. The dialysis method also involves the use of titration (18, 27, 28).

During the past decade continuous determination of the intragastric pH by glass electrode has come into increasing use. This investigation appears to be particularly suited for assessing the effect of drugs. In an investigation of the pH of a solution, the glass electrode as well as the reference electrode are generally placed in the solution whose pH is to be measured. In the intragastric determination of pH on patients several investigators have placed the reference electrode on the rectal mucosa, oral mucosa (2, 16, 17, 30) or on the skin (8, 15, 21, 24, 25, 26) instead of having both electrodes in the stomach (6, 7, 9, 23, 24). Below the technique and accuracy of the skin electrode method will be described.

Technique

For determining the pH of gastric juice *in situ* the author used a glass electrode (G282/Sp Radiometer) 2 cm in length and 3 mm in diameter. The recording surface of the glass electrode is 70 mm². This glass electrode is introduced through the patient's nose into the stomach. Its wire, 70 cm in length, is copper wire embedded in polyethylene and has a total diameter of 1 mm. That part of the tubing which is not within the patient consists of a lead-shielded cable.

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diate cause of death This as well as the circulatory disturbances of fingers and toes were caused by thrombotic occlusion of small vessels owing to a reaction to the deposits of crystals Probably, an increased oxalate deposition in the arterial walls as the result of the intravenous calcium injections might have taken place The administration of sympathomimetic drugs might also have been of importance In animal experiments epinephrine injections may cause deposits of calcium salts in the vessel walls (4)

The clinical signs of myocardial damage are accounted for by the deposits of crystals in the heart muscle

The past history with the demonstration of renal stones in infancy together with the finding of nephrocalcinosis by roentgenological examination, might have given clues to the correct diagnosis A re-examination of sections from the kidney removed 22 years earlier might also have been of value However, the clinical course with a symptom free interval of more than 20 years deviates definitely from previously reported cases The presence of consanguinity could *also have been a diagnostic key* Consanguinity has been described previously in three families with oxalosis (3)

There is no specific therapy available Omitting oxalate containing food from the diet is without effect Nor are there any means by which the renal excretion of oxalate can be augmented

Summary

The case of a 26-year-old woman with oxalosis is reported There was consanguinity between her parents At the age of 4 a nephrectomy was performed because of renal calculi She was then entirely free from symptoms for 22 years when she developed uremic symptoms A complete heart block and peripheral skin necroses were prominent clinical features She died of acute pancreatitis The diagnosis was established at autopsy by the demonstration of disseminated calcium oxalate monohydrate deposits A special feature was the thrombotic occlusion of small arteries, probably caused by crystal deposits

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fluid bridge were compared only when this reference electrode had been used for less than 4 minutes before or after the aspiration

The three sets of electrodes were controlled immediately before and after each investigation against 0 05 M potassium tetraoxalate which at 25 C has a pH of 1 68 and at 35° C a pH of 1 69 The buffer adjustment of G200 and K100 used in measuring the pH of aspirated samples of gastric juice was done at 25 C The other sets of electrodes (G282 + KS62084 and G282 + K101), used in determining intragastric pH were buffer adjusted at 35° C

The temperature in the stomach varies under the influence of histamine and anticholinergic drugs maximally from 36 5 to 38 5° C (4) The temperature compensator of the pH meter was set at 35 C during the continuous determination of intragastric pH Then the difference in temperature can at most cause an error of 0 02 in the pH reading

In cases where a high pH was expected the adjustment was also carried out in a phosphate buffer of pH 6 5 In order further to secure a correct adjustment a 0 0994 N hydrochloric acid which has a pH 1 1 was used at intervals

The position of the glass electrode in the antrum which makes up the proximal area of the pyloric part was controlled radiographically at the beginning of the pH determination The radiography was repeated in the event of unexpected fluctuations in the pH and at the termination of the investigation if it had lasted for more than 2 hours

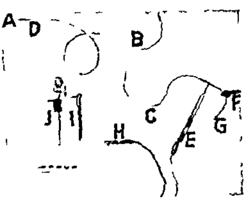


Fig 2 The electrodes used A The glass electrode (G282/Sp) for intragastric determination B Its wire C Plastic tube D Openings in the plastic tube for aspiration of gastric juice E Reference electrode (K101) with its tip in the plastic tube F Binding screw G Needle H Reference electrode (KS62084) to be placed on the skin I Glass electrode (G200) and J Reference electrode (K100) both for determination in aspirated gastric juice

Material

The material comprises 35 patients admitted for dyspepsia or pernicious anaemia They were not classified by diagnosis The patients were fasting during the investigation which lasted for about 90 minutes In two patients who were receiving oxiphenonium bromide (NFN) (Antrenyl®) however it was continued for 5 hours Four patients were excluded I two because the glass electrode was not adjustable at the end of the investigation One patient with gastric carcinoma showed an extremely irregular curve which could not be compared with the results of the studies on the other patients The irregularity of the curve was presumably due to an insufficient mixing of occasionally haemorrhagic secretion One patient was excluded because he was given a glass of water owing to a lack of secretion

After 30 minutes determination of pH *in situ* 20 of the remaining 31 patients received injectable histamine 1 ml (1 mg/ml) In 4 cases however the dosage was reduced because of heart disease Ten of the patients who received histamine had — also before the injection — free acid in the stomach Two patients were injected intramuscularly with 1 ml oxiphenonium bromide (NFN) (Antrenyl®) (2 mg ml)

The reference electrode (KS62084) on the skin was used uninterruptedly in 13 patients in determining intragastric pH In the remaining 18 patients the author alternated between the reference electrode (KS62084) on the skin and the reference electrode (K101) in the fluid bridge

For purposes of comparing the pH *in situ* and in aspirated samples of juice each patient had an average of 6 samples taken The pH in the sample was compared in 145

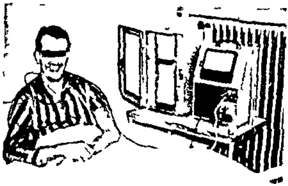


Fig 1 *A patient during continuous pH determination* by a glass electrode in the stomach and a reference electrode on the upper arm The wire of the glass electrode is fixed to the patient's cheek at the junction of the polyethylene coated copper wire and the lead shielded cable The wires of the glass and reference electrodes are connected to a pH meter and recorder

The reference electrode is a *calomel electrode* with saturated potassium chloride and a porous plug (K562084, Radiometer) which is fixed by *adhesive tape to the patient's upper* arm The wires from the two electrodes are connected, *via* a pH meter (Radiometer), to a *recorder (type* REC 1, *Radiometer) which* continuously records the pH on paper by an ink writer In the present investigations, the *paper was carried at a rate of 12 cm/hour* The pH interval extends from e g pH 1 to pH 8 (1 pH corresponding to 1 cm) (fig 1)

*In order to make it possible to compare in*tragastric pH with the pH in aspirated samples of gastric juice a soft plastic tube, of *an internal diameter of 1 5 mm* and a wall thickness of 0 5 mm was fixed to the proximal end of the glass electrode The two apertures of the plastic tube for aspiration of gastric juice were at a distance of 2 1/2 cm from the ball of the glass electrode At short intervals, the wire of the glass electrode and the plastic tube were tied together with a nylon thread The tube as well as the wire were of easily flexible material to reduce the risk of the glass electrode being pressed against the gastric wall Owing to its slight weight the glass electrode floated free in the gastric contents

Since the method showed a higher pH *in situ* than in the aspirated sample of juice it was investigated whether this difference could be explained by the placement of the reference electrode on the skin To this end, the following procedure was employed Through a short side tube close to the oral end of the plastic tubing, the tip of a reference electrode (K101, Radiometer) was placed in the lumen of the plastic tube After completing each aspiration, the free opening of the tube was closed by a binding screw peripheral to the named side tube Thus *via* the fluid column in the tube, the reference electrode was directly connected with the juice in the stomach Under these circumstances, the glass electrode in the stomach and the reference electrode in the fluid bridge (i e the gastric juice in the plastic tube) were in the same secretion only the distance between them was 60 cm When the plastic tube was filled with juice, it was possible, during the continuous determination of intragastric pH, to use alternately the reference electrode on the skin and the reference electrode in the fluid bridge by merely exchanging the wires of the two reference electrodes in the pH meter Exchange of the reference electrode was carried out at a few minutes' intervals However, the reference electrode in the fluid bridge was applicable only when there was ample secretion in the stomach Otherwise, the aspiration resulted in the escape of gastric gas into the fluid bridge, and the gas disturbed the determination of the pH

During the continuous measurement of intragastric pH, 1—3 ml gastric juice was aspirated with a 10 ml syringe approximately every 10 minutes The entire gastric contents were aspirated only when a drug was administered during the investigation and at its termination So that the determination of intragastric pH could be done undisturbed during the sampling of the gastric juice, the latter was carried out when the reference electrode on the skin was connected The pH of the sample was determined immediately after the aspiration by a glass *electrode* (G200) and reference electrode (K100) and compared with the pH measured in the stomach at the moment *of aspiration (fig 2)*

The pHs in aspirated juice and *in situ* during use of the reference electrode in the

flu d bridge were compared only when th s reference electrode had been used for less than 4 m nutes before or after the asp rat on

The three sets of electrodes were controlled immed ately before and after each invest ga t on aga nst 0 05 M potass um tetraoxalate wh ch at 25 C has a pH of 1 68 and at 35° C a pH of 1 69 The buffer adjustment of G200 and K100 used in measuring the pH of aspira ed samples of gastric ju ce was done at 25 C The other sets of electrodes (G282 + KS62084 and G282 + K101) used in deter m n ng intragastr c pH were buffer adjusted at 35° C

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Material

The material comprises 35 pat ents adm tted for dyspeps a or pern cious anaemia They were not class f ed by d agnosis The pat ents were fas ng d r ng the in est gat on wh ch las ed for about 90 m nutes In two pat ents who were rece ng ox phenon um brom de NFN (Antrenyl®) however t was cont n ed for 5 hours Four pa ents were excluded T wo because the glass electrode was not ad jus able at the end of the in est gat on One patient w th gas ric carcinoma showed an ex tremely irregular c rve wh ch could not be

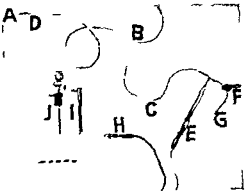


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The reference electrode (KS62084) on the *skin* was used un nterruptedly in 13 patients in determ n ng intragastric pH In the rema n ng 18 pat ents the author alternated between the reference electrode (KS62084) on the sk n and the reference electrode (K101) in the flu d bridge

For purposes of comparing the pH *in s tu* and in asp rated samples of ju ce each pa t ent had an average of 6 samples taken The pH in the sample was compared in 145

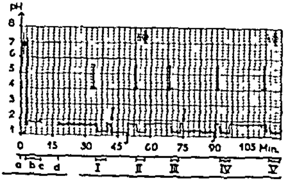


Fig 3 Continuous recording of intragastric pH Abscissa Time in minutes Ordinate pH The reference electrode on the skin was used initially From the 40th to the 50th minute and from the 75th to the 90th minute, it was replaced by the reference electrode in the fluid bridge, marked ⌐⌐ a The glass electrode *en route* through the patient's nose and oesophagus where the pH was 6 5—7 Four minutes later there was an abrupt fall in pH to 1 5, when the glass electrode reached the fundus of the stomach b Within 10 minutes the glass electrode proceeded further through the stomach due to peristaltic motility During this time the pH rises to 1 8 and later to 2 c The glass electrode close to the pylorus d The glass electrode in the antrum after it had been retracted 4—5 cm under fluoroscopy, this resulted in a fall of pH of 0 3 In the 34th minute of the test, the first sample was aspirated The pH in the sample was determined at 1" Two minutes later the reference electrode in the fluid bridge replaced the reference electrode on the skin for a period of 10 minutes, etc

cases, with pH *in situ* using the reference electrode on the skin and in 60 cases with the pH *in situ* using the reference electrode in the fluid bridge

Exchange between the two reference electrodes during the pH measurement in the stomach was carried out 59 times

Results

1) pH determined *in situ* using the reference electrode on the skin 2) using the reference electrode in the fluid bridge and 3) pH determined in aspirated samples of gastric juice, often do not show conformity For studying the gastric acidity the pH determined *in situ* with the reference electrode in the fluid bridge is more correct Therefore, with the exception of cases having sparse secretion, this method formed the basis of dividing the patients into 3 groups In exceptional cases the pH determined *in situ* with the reference electrode on the skin formed the basis of the grouping

I *Nineteen patients with free acid in* the stomach, i e pH < 3.3 before as well as after administration of histamine To these may be added the two patients who had free acid in the stomach prior to the administration of oxiphenonium bromide (NFN) II Three patients with histamine-refractory achlorhydria, *i e* pH *in situ* > 6.3 before histamine and > 5.8 after histamine (all three were suffering from pernicious anaemia) III Seven patients with primary, not histamine-refractory achlorhydria, i e pH around the neutral point before histamine was administered, but < 3.3 after its administration

pH measured in the gastric juice by the three different procedures was compared, as far as possible, within each of the three patients groups In the patients with primary, non histamine refractory achlorhydria the comparison with pH determined *in situ* with the reference electrode in the fluid bridge could not be done because of too small a quantity of secretion to form a fluid bridge As the corresponding comparisons in patients with histamine refractory achlorhydria were so few, the results are only given schematically and were not submitted to a numerical analysis

a pH in the various parts of the stomach

As the glass electrode was being passed through the patient's nose and oesoph-

agus to the antrum, alterations in pH were observed

1) In patients with free acid in the stomach the pH fell by 5—6 when the tube passed from the oesophagus into the stomach During its passage through the stomach the alterations were less marked, the pH in the fundus usually being 0 2—0 5 lower than in other parts of the stomach

2) Patients with histamine refractory achlorhydria had a pH close to neutral in all parts of the stomach and in the oesophagus

3) In patients with primary non histamine refractory achlorhydria the glass electrode usually passed an area with a pH of less than 3 corresponding to the fundus while in other parts of the stomach the pH was close to neutral (histamine was given 30 minutes later)

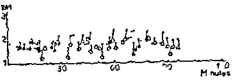


Fig 4 Intragastric pH determined with a reference electrode on the skin (●) pH in aspirated sample of gastric juice (○) The intragastric pH of 9 patients is marked every 5th minute and compared in 39 cases with the pH in the samples of aspirated juice Abscissa Time in minutes. Ordinate pH The line between (●) and (○) indicates the magnitude of the difference *between pH in situ and in the sample* minimum 0 05 maximum 0 6

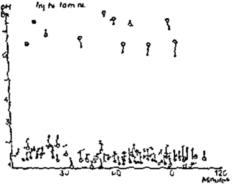


Fig 5 Intragastric pH determined with a reference electrode on the skin (●) pH in aspirated sample of gastric juice (○) The intragastric pH of 13 patients marked every 5th minute before as well as after administration of histamine and compared in 67 cases with the pH in samples Abscissa Time in minutes Ordinate pH The line between (●) and (○) indicates the difference between pH in situ and in sample minimum 0 and maximum 0 7 in 10 patients who had free acid and whose results are stated at the bottom of the figure Only 5 of these patients received histamine At the top the results found in 3 patients with histamine refractory achlorhydria

b Comparison between pH in aspirated samples of gastric juice and pH determined in situ using the reference electrode on the skin

1) In 19 patients with free acid in the stomach the pH *in situ* proved higher than in the sample In 9 of these patients, on whom 39 comparisons were made the difference ranged from 0 05 to 0 6 pH average 0 31 pH *Histamine* administered to the remaining 10 patients *did not definitely increase the difference* In 56 comparisons the difference in these patients was a minimum of 0, a maximum of 0 7 and averaged 0 35 pH

The increase in pH induced by oxiphenonium bromide (NTN) (Antrenyl®) administered to two patients with free acid in the stomach took place more rapidly *in situ* than in aspirated juice A conformity was found again after the pH *in situ* had been stationary (around 6 5) for 75 minutes This assessment is based on 18 comparative investigations

2 While in patients with free acid in the stomach the pH was higher in situ

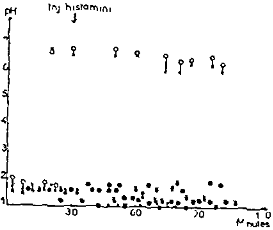


Fig 6 Intragastric pH determined with a reference electrode in a fluid bridge (x) pH in aspirated sample of gastric juice (○) The gastric pH of 11 patients marked every 5th minute and compared 60 times with the pH in aspirated secretion Abscissa Time in minutes Ordinate pH ⊗ indicates coincidence of the two values A difference between pH in situ (x) and in aspirated sample (○) is indicated by a connecting line For 10 patients with free acid in the stomach this difference is stated at the botton of the figure, a maximum of 0 1 pH However, in 3 cases with a very mucous secretion there was a greater difference, indicated by a transverse line At the top the results found in one patient with histamine refractory achlorhydria This patient and 5 of the others received histamine

than in the aspirated samples, the reverse applied to patients with histamine refractory achlorhydria The individual results of 11 comparative investigations are given in fig 5

3) The 7 patients with primary, non-histamine refractory achlorhydria were studied by 21 comparative investigations This showed that as a rule the pH *in situ* was lower than in aspirated samples of juice unless histamine had induced a pH < 2 6 in the stomach This fact must be borne in mind and it should also be remembered that some of these patients had sparse and sometimes periodically no secretion to aspirate

This is the reason why according to the pH determination in aspirated samples 2 of the 7 patients would be grouped as histamine-refractory achlorhydrias, although the concurrent, continuous determination of pH *in situ* revealed secretion of acid after histamine

c Comparison between pH in aspirated samples of gastric juice and pH determined in situ using the reference electrode in the fluid bridge

As already mentioned the difference between pH in aspirated samples of gastric juice and *in situ* seemed to be less marked when the reference electrode in the fluid bridge was used in the intragastric determination

1) In 10 patients with free acid in the stomach it was found, in 51 comparisons, that the pH in the samples was usually equal to the pH *in situ* Apart from one patient with grossly mucous secretion prior to a full histamine effect, the pH *in situ* was a maximum of 0 1, and on an average only 0 04 higher than in the samples

2) In 9 comparisons between pH in samples and *in situ* using the reference electrode in the fluid bridge in one patient with histamine-refractory achlorhydria the pH was lower *in situ* The difference was less marked than that found with the reference electrode on the skin

d pH in situ when altering the technique of determination

During the continuous determination of intragastric pH the use of the reference electrode (KS62084) on the skin and of the reference electrode (K101) in the

fluid bridge of gastric juice was alternated at a few minutes intervals This change-over resulted in an alteration in pH

1) pH proved lower in 10 patients with free acid in the stomach when the reference electrode in the fluid bridge was connected, instead of the reference electrode on the skin to the pH meter Change of reference electrodes was done 51 times Alterations in pH following this change ranged from 0 2—0 7, averaging 0 37 These alterations were *not* influenced by histamine, which was administered to 5 of the patients

2) Conversely in one patient with histamine refractory achlorhydria the pH was higher when the reference electrode in the fluid bridge was used in determining intragastric pH

e Fluctuation in the record of the intragastric pH

Without preceding medication there were at times fluctuations in the pH of 1—6 during the continuous intragastric determination By aspiration of secretion and change of the reference electrodes the e fluctuations were found to be caused by (I) Reflux of bile stained duodenal contents (II) Distension of the stomach with air so that the glass electrode was situated in air instead of in gastric juice III) Sparse secretion in the stomach so that the mucous membrane of the pyloric part was covered mainly by its own alkaline secretion IV Lack of or insufficient contact etween the reference electrode and the kin V Bubbles of air in the gastric uice in the plastic tube while this juice was serving as a medium for the reference elec rode

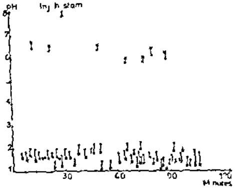


Fig 7 Intragastric pH determined with a reference electrode on the skin (●) Intragastric pH determined with a reference electrode in a fluid bridge (x) 59 double determinations of pH in situ Abscissa Time in minutes Ordinate pH The bottom of the figure represents 10 patients with free acid in the stomach The length of the connecting line from (●) to (x) indicates that the pH in situ is 0 2—0 7 lower *using the reference electrode in the fluid bridge than using* the reference electrode on the skin At the top the results in one patient with histamine refractory achlorhydria In this case the pH was higher with the reference electrode in the fluid bridge

Discussion

The pH determined in aspirated gastric juice and *in situ* has previously been compared in *patients with free acid in the stomach* Apart from Marcussen and Rygvold (16) the various authors (6 7, 9 24) have found a lower pH in aspirated gastric juice than *in situ* In the present study the pH in aspirated samples was also found to be lower than in situ although the difference was almost obliterated when the reference electrode in the fluid bridge was used during the *in situ* determination

When the glass electrode is placed in the fundus of the stomach and the reference electrode on the oral mucosa as was done by Marcussen and Rygvold the pH recorded *in situ* is lower than with

the respective electrodes in the antrum and on the skin. This partially explains these authors' findings.

That the pH is lower in the fundus than in other parts of the stomach has been shown by Tomenius and Williams (30) as well as by the present study. Accordingly, the placement of the glass electrode must be known, and maintained, if the study is to compare (1) pH *in* aspirated secretion and *in situ*, (2) intragastric pH in different patients, or (3) pH *in situ* in the same patient under basal conditions and under the influence of gastric drugs.

As has been demonstrated, the difference between pH *in situ* and in aspirated samples was appreciably reduced, and the pH *in situ* altered, when the reference electrode in the fluid bridge replaced the reference electrode on the skin. This indicates that the determination of pH *in situ* with the reference electrode on the skin is disturbed by an electrical potential difference, which is excluded by using the reference electrode in the fluid bridge. By two calomel electrodes, such a potential has been measured directly between skin and gastric mucosa (11, 22, 24). Investigations on frogs and dogs have shown that it is made up predominantly of a potential between the gastric mucosa and peritoneal surface and that it is not influenced by an increased rate of acid secretion (3, 10). In a determination of intragastric pH with a glass electrode in the stomach and a reference electrode on the skin, the potential between skin and gastric mucosa is added to the potential which is due to the presence of free hydrogen ions in gastric juice, and thereby it will be included in the reading of the intragastric pH. An indirect measure of the size and direction of the potential between skin and gastric mucosa is obtained by recording the alteration in the gastric pH, when the reference electrode in the fluid bridge replaces the reference electrode on the skin.

It is agreed that the pH in patients with free acid, as shown in fig. 7, is recorded 0.2—0.7 too high, when the named potential is included. On the other hand, only the studies of Ingram and Ricards (11) indicate that the potential between skin and gastric mucosa is oppositely directed in *patients with histamine refractory achlorhydria*. Owing to this direction of the potential, the pH *in situ*, determined with a reference electrode on the skin, is recorded too low in these patients (fig. 7).

Even when the disturbing potential was excluded by using the reference electrode in the fluid brigde, the pH in patients with histamine-refractory achlorhydria was higher in aspirated secretion than *in situ* (fig. 6). A plausible explanation of this phenomenon may be found in the publication of Kahn and Stokes (13). As gastric juice having a pH exceeding 5.5 is exposed to atmospheric air instead of the carbon dioxide containing gastric air, carbon dioxide will diffuse from the juice whose pH consequently will increase.

Ample, visible mucus was observed in one patient, who also had free acid in the stomach, and in the majority of the patients with achlorhydria. Gastric mucus has a buffer capacity. This fact, combined with a poor mixing of gastric juice *in situ* (6) influences the difference be-

tween pH determined in aspirated gastric juice and *in situ* As the sample of gastric juice is aspirated, its mixing becomes more complete, and this improves the possibility of the acid binding capacity of the mucus Thus, in the above mentioned patient with free acid and ample mucus, the pH was higher in the sample than *in situ*, when the latter was measured with the reference electrode in the fluid bridge

In a similar manner, the influence of mucus at times manifested itself in patients with primary, non histamine refractory achlorhydria The increase in pH during the aspiration masked, in two patients a sparse histamine induced acid secretion which consequently could be demonstrated only by determination of the pH *in situ* Thus determination of pH *in situ* is well suited to distinguish hypochlorhydria from histamine refractory achlorhydria The latter was found in only 3 of the present patients, all of whom had pernicious anaemia

At the time of an incipient effect of oxiphenonium bromide (MTN) the incomplete mixing of the gastric juice manifested itself as an increased difference between pH determined in aspirated samples of gastric juice and *in situ*

The determination of the pH is less affected by the conditions at the site of the liquid junction i e the junction between the saturated potassium chloride bridge of the reference electrode and the solution whose pH is to be determined A potential here liquid junction potential of unknown magnitude causes an inaccuracy in the determination of pH in gastric juice as well as in the determination of pH on the whole It is not definitely known whether the presence of mucus in the gastric juice influences the magnitude of the liquid junction potential (12, 19)

In determination of the pH *in situ*, with the reference electrode in the fluid bridge (that is, the gastric juice in the plastic tube), the temperature at the liquid junction was about 10° C lower than in the stomach This influences the liquid junction potential so that the pH *in situ* was recorded 0 01 too high (29) Siggaard Andersen (29) also found that at a continuous pH measurement the liquid junction potential depended upon how often the liquid junction was renewed If this was done every 15 minutes the pH altered only by 0 003 when the fluid surfaces were renewed In the present investigations, aspiration of samples of the gastric juice for comparison of pH in sample and *in situ* caused such a renewal of the liquid junction at a few minutes intervals

The difference between pH in aspirated samples of gastric juice and *in situ* using the reference electrode in the fluid bridge can only be influenced by the two last mentioned, known quantities

The reference electrode placed on the skin had a porous plug unlike the other two reference electrodes which had a free fluid surface With the use of a reference electrode with a porous plug, the pH is recorded 0 02 too high (29)

Since pH is a logarithmic quantity, these small divergences in the pH are also of significance At a low pH they may represent a considerable number of acid equivalents (20)

In clinical practice it is of interest to rule out the function of proteolytic en

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It is agreed that the pH in patients with free acid, as shown in fig 7, is recorded 0 2—0 7 too high, when the named potential is included On the other hand, *only the studies of Ingram and Ricards* (11) indicate that the potential between skin and gastric mucosa *is oppositely* directed in *patients with histamine refractory achlorhydria* Owing to this direction of the potential, the pH *in situ*, determined with a reference electrode on the skin, is recorded too low in these patients (fig 7)

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It is concluded that determination of pH *in situ* is particularly well suited for assessing the effect of diets and of anticholinergic drugs as well as antacids thanks to the gentleness of the procedure and the fact that it does not require aspiration It is stated that determination of pH *in situ* using a fluid bridge is more correct

It is anticipated that determination of intragastric pH using a reference electrode on the skin will replace the usual investigation of the fasting secretion collected by a duodenal tube

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Conclusion

Measurement of intragastric pH has afforded a unique opportunity of assessing the effect of diets, antacids, and anticholinergic drugs upon the gastric concentration of free acid Thanks to the negligible discomfort to the patient, the investigation may be extended over several hours, and it does not necessarily require sampling

The accuracy of the method could be increased by placing the reference electrode in a fluid bridge of gastric juice instead of on the skin This procedure, which involved somewhat more discomfort to the patient, secured registration of alterations in pH *in situ* of less than 0 1 Even a very slight effect of e g an anticholinergic drug, will thus be revealed by a pH determination *in situ*

Even with the reference electrode placed on the skin, determination of pH *in situ* proved to be superior to the aspiration method in respect to the differentiation between patients having free acid in the stomach, patients having histamine-refractory achlorhydria, and patients having primary, non histamine refractory achlorhydria As the procedure is also extremely gentle it will probably gradually replace the usual investigation of fasting secretion by duodenal tube

Summary

Gastric acidity was studied in 35 patients by continuous measurement of the pH by a glass electrode (G282/Sp, Radiometer) in the stomach and a reference electrode (KS62084, Radiometer) on the skin The wires of the electrodes were connected *via* a pH meter 22 (Radiometer) to a recorder (RI C 1, Radiometer) The investigations were carried on for an average of 90 minutes — in two patients, who were on oxiphenonium bromide medication, however, for 5 hours

Determination of intragastric pH is a very gentle procedure, and the object was to assess the accuracy of the method For this purpose, samples of gastric juice were aspirated at a *few minutes'* intervals and their pH compared with the pH determined *in situ* at the moment of sampling

According to the determination of intragastric pH the patients were divided into three groups (1) Patients with free acid in the stomach, (2) patients with histamine refractory achlorhydria, and (3) patients with primary, non histamine refractory achlorhydria

In patients with *free acid in the stomach* 95 comparisons revealed that the pH in aspirated secretion was an average of 0 33 lower than the pH *in situ* Conversely, in patients with achlorhydria the pH in the aspirated secretion was higher than that *in situ*

Various causes of the difference between pH determined in aspirated samples of gastric juice and the pH *in situ* are discussed The main cause proved to be an electrical potential difference between skin and gastric mucosa This potential is included in the recording of pH, when the reference electrode is placed on the skin Determination of pH *in situ* using a reference electrode in direct fluid connec

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It is concluded that determination of pH *in situ* is particularly well suited for assessing the effect of diets and of anticholinergic drugs as well as antacids, thanks to the gentleness of the procedure and the fact that it does not require aspiration It is stated that determination of pH *in situ* using a fluid bridge is more correct

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Aus dem König Gustaf V s Forschungsinstitut Stockholm Schweden

Untersuchungen an mittels Rivanol gewonnenen Serumfraktionen

Von

HELMUT SCHATZ[1]

Horejši und Smetana beschrieben im Jahre 1956 in dieser Zeitschrift (3) eine Methode zur Isolation von Gamma globulin aus Blutserum durch Rivanol (= 2 Äthoxy 6 9 diaminoacridinlactat) womit sie eine Gammaglobulinanreiche rung bis zu 98 % erzielten der Rest be stand aus Beta und manchmal Alpha globulinen Mit gewissen Rivanolpräpa raten betrug bei ihnen die Betaglobulin beimengung bis zu 20 % Boettcher et al (1) fanden in der Rivanolfraktion einen relativ hohen Transferringehalt und ver wendeten diese als Ausgangsmaterial Isolierung dieses Proteins Zu gleichen Ergebnissen kamen auch andere Auto ren (5 18) Steinbruch und Quentin stellten das Coeruloplasmin durch zwei fache Rivanol Subfraktionierung aus den Fraktionen III und IV nach Cohn dar 12 auch das α_2 Makroglobulin isolier ten sie unter Zuhilfenahme von Rivanol 13 Laurell (7) beschrieb in der nach der Originalmethode gewonnenen Rivanol fraktion neben dem 7S Gammaglobulin und Transferrin noch Spuren von relativ polysaccharidreichen α_1- α_2 und β_1 Komponenten

In Hinblick auf die Einfachheit der Methode war es von Interesse zu wissen, ob in der Rivanolfraktion auch das γ_1 Makroglobulin ($-\beta_{2M}-$ 19S-Gam maglobulin) enthalten sei, da wir sie dann eventuell zur Gewinnung des den γ_1 Makroglobulinen zugehörigen Rheu mafaktors hatten heranziehen können Überhaupt erschien es wichtig festzu stellen ob die mit Rivanol isolierten Gammaglobuline das gesamte Gam maglobulinkomponentensystem (γ_{1A}, γ_{1M} und γ_2 Globulin) bzw alle drei Immunoglobuline (β_{2A}, β_{2M} und γ Globulin) umfassten Aus der zugang lichen Literatur wurde nur ein indirekter Hinweis in dieser Richtung bekannt Laurell (7) verwendete die Fraktionier ung mit Rivanol zur Darstellung von Paraproteinen (= M Komponenten) und fand dass von den verschiedenen im munologischen Typen der M Kompo nenten die γ_{1A} und γ_{1M} Paraproteine zum Unterschied von den dem γ_2 Typ

Bei der Redaktion am 11 September 1964 eingegangen
[1] Derzeitige Adresse II Med Univ Klinik Allgemeines Krankenhaus Wien Österreich

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TABELLE I

Fraktion	pH	μ
a	8 5	0 25
b	8 5	0 20
c	7 3	0 15
d	7 3	0 05
e	7 3	0 01

zugehorigen, die gewohnlich in Losung blieben, regelmassig prazipitiert wurden

Die folgenden Untersuchungen wurden im Laboratorium von Prof Nanna Svartz im obengenannten Institut durchgefuhrt

Material und Methoden

Normalserum wurde von der Blutspenderzentrale des Karolinska Sjukhuset Stockholm Serum von an *rheumatoider Arthritis* leidenden Patienten von Prof Nanna Svartz Konung Gustaf V s Forskningsinstitut Stockholm freundlicherweise uberlassen

Die *Rivanolfraktionierung* wurde nach Horejsi und Smetana (3) vorgenommen wobei die vom Prazipitat und vom Rivanol befreite supernatante Flussigkeit durch Vakuumdialyse gegen eine 0 9 %ige NaCl Losung auf das Serumausgangsvolumen eingeengt wurde

Die *Immunelektrophorese* wurde in der Mikromodifikation nach Scheidegger (10) die *Agargelprazipitation* in der Modifikation nach Wadsworth (16) durchgefuhrt Die verschiedenen *Antiseren* wurden teils selbst hergestellt teils vom Zentrallaboratorium des Bluttransfusionsdienstes des Niederlandischen Roten Kreuzes im Amsterdam bezogen

Fur die *freie Elektrophorese nach Tiselius* ermoglichte uns Herr Laborator Dr B Olhagen Vorstand der Rheumatologischen Klinik des Karolinska Sjukhuset Stockholm die Anwendung *einer LKB Apparatur Type 3021 B*

Bei der *Mikroagargelelektrophorese* folgten wir den Angaben von Wieme (18)

Zur *analytischen Ultrazentrifugierung* stellte Prof Nanna Svartz ihre Anlage zur Verfugung (Spinco Model E analytischer Rotor Typ D 59780 RPM)

Der *Waaler Rose Test* zur Bestimmung des Rheumatoidfaktors wurde in der Modifikation von Svartz (14) vorgenommen

Ergebnisse

In vorausgehenden Versuchen wurde festgestellt, dass fur die Fraktionierung vor allem die Jonenstarke der verwendeten 0,4 %igen Rivanollosung von ausschlaggebender Bedeutung ist, weiters spielt der pH eine sehr grosse Rolle

Praktisch unverandert in ihrem Loslichkeitsverhalten blieben im gesamten angewandten Bereich (pH von 5,8 bis 10,0, μ von 0,01 bis 0,35) das γ_2 und γ_{1A} Globulin, das Transferrin und das Hamopexin (15), die jedesmal in uberwiegendem Teil im Uberstand enthalten waren, wahrend das γ_1 Makroglobulin nur in einigen wenigen Fallen und dann nur in geringsten Spuren nachweisbar war Das Albumin und die verschiedenen α_1 und α_2 Globulinfraktionen wurden bei abnehmender Ionenstarke und bzw oder steigendem PH in fortschreitendem Ausmasse prazipitiert Die Abb 1 bis 3 zeigen einige ausgewahlte in Tab I charakterisierte Fraktionierungsergebnisse

In der freien Elektrophorese nach Tiselius zeigten sich in Fraktion d (pH = 7 3 μ 0 05) lediglich β und γ Globulin und zwar in einem Verhaltnis von 27 zu 73 % Die analytische Ultrazentrifugierung derselben Fraktion ergab auch bei einem Aufnahmewinkel von

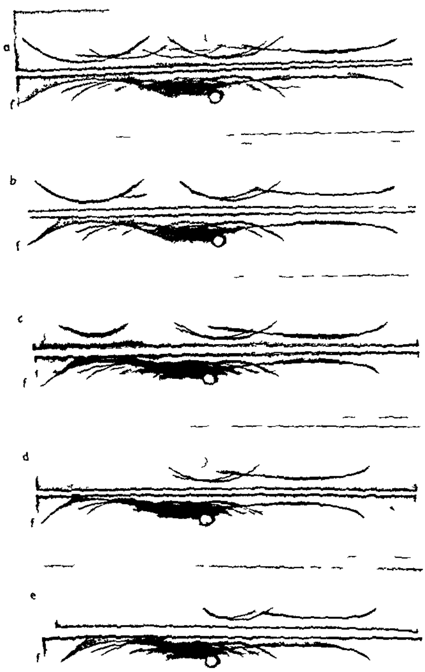


Abb. 1 ... elek...ph...rese...n ... An... Humanseru... a ... R... anolfakt... onen (s...ehe Tab. 1) f ... N... r...alse...u...

... Helmut Schatz

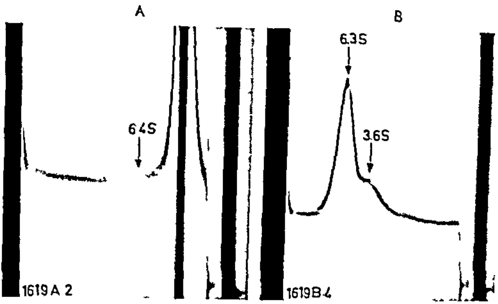


Abb. 4 Analytische Ultrazentrifugierung, Sedimentationskonstanten unkorrigiert. A: 18 Min. nach Erreichung von 59780 RPM, Aufnahmewinkel 35°. B: 105 Min. nach Erreichung von 59780 RPM, Aufnahmewinkel 55°

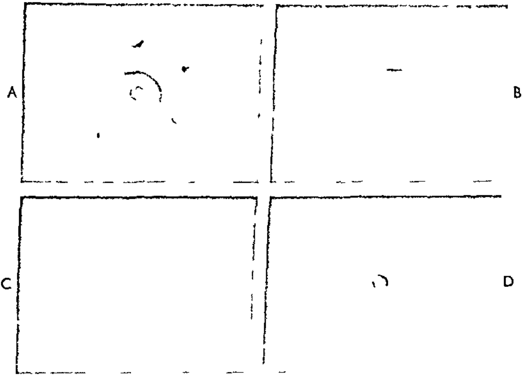


Abb. 5 Agargelpräzipitation. Verdünnungsreihen der Rivanolfraktion beginnend Mitte oben im Uhrzeigersinn 1:1, 1:2, 1:4, 1:8, 1:16, 1:32. Mittleres Loch: Antiseren A: [illegible], B: Serum 1:4; C: [illegible] Fraktion von unabsorbiertem Serum, D: [illegible] Fraktion von absorbiertem Serum

35° keine 18—19S Zacke Es zeigten sich geringe Mengen von 6,4S Material und als Hauptanteil 6,3S- und 3,6S Proteine (Werte unkorrigiert Abb 4) Die erste Zacke ist nach dem immunelektrophoretischen Befund dem γ_{1A} Globulin zuzuordnen während die beiden folgenden dem γ_2 Globulin bzw Transferrin und Hamopexin entsprechen Fur ihr Verhaltnis wurden dieselben Werte errechnet, wie sie sich auch in der freien Elektrophorese ergeben hatten

Bei Serum von an rheumatoider Arthritis leidenden Patienten fiel im Hamagglutinationstest mit sensibilisierten Schaferythrozyten der auf die Anwesenheit des Rheumafaktors zuruckfuhrbare Titer von 1 4096 bzw 1 2048 jeweils auf Null ab

In den Fallen, wo die Rivanolfraktion von nicht mit normalen Schaferythrozyten zur Absorption der unspezifischen Heteroagglutinine behandelten Rheumatikerserum gewonnen wurde, blieb gelegentlich ein Titer von 1 32 erhalten Diese Befunden entsprach auch das Ergebnis der Agargelpräzipitation mit spezifischem γ_{1M} Antiserum (Abb 5) wobei die Rivanolfraktion von absorbiertem Serum (D) nicht die geringste Spur einer Prazipitationslinie ergab wahrend bei Verwendung von nicht absorbiertem Serum (C) γ_{1M} Globuline in der niedrigsten Verdunnungsstufe nachweisbar waren

Diskussion

Die Serumauftrennung mit Rivanol ist zweifellos eine elegante und einfach anwendbare Methode das dabei gewonnene Gammaglobulin ist nach den Untersuchungen von Horejsi und Smetana (3) weder physikochemisch verändert noch von geringerer biologischer Aktivitat als die nach Cohns Äthanolmethode hergestellte Fraktion Zur Erzielung eines elektrophoretisch reinen Gammaglobulins verwendeten die obgenannten Autoren noch eine Subfraktionierung mit Athanol in Kalte

Nach unseren Untersuchungen enhält die Rivanolfraktion weitgehend unabhängig von angewandtem pH und Ionenstarke von den drei Immunoglobulinen stets zwei in hoher Konzentration, namlich das γ_{1A} und das γ_2 Globulin Das γ_{1M}- Globulin jedoch wird fast immer zur Gänze präzipitiert und verbleibt nur gelegentlich in geringsten Mengen in der Fraktion gelost Insbesondere trifft dies auch fur den den γ_{1M} Globulinen angehorigen Rheumafaktor zu

Unser Befund, dass von den Immunoglobulinen neben dem γ_2 Globulin auch das γ_{1A} Globulin immer in Losung bleibt, ist insoferne interessant als Laurell (7) mit Rivanol eine Prazipitation der zum γ_{1A} Typ gehorigen Paraproteine beobachtete Eigene Erfahrungen mit γ_{1A} M Komponenten liegen nicht vor und es muss die Moglichkeit diskutiert werden dass sich das normale γ_{1A} Globulin in seinem Loslichkeitsverhalten gegenuber Rivanol von den ihm immunologisch entsprechenden Paraproteinen unterscheidet wahrend die γ_{1M} und γ_2 Paraproteine in dieser Hinsicht ihren immunologischen Vorbildern gleichen Ein dazu paralleles Verhalten ware ubrigens auch im Hexosegehalt zu erblicken, der bei γ_{1A} M Komponenten nach Laurell und Heremans (8) betrachtlich nie-

driger als bei normalen γ_{1A}-Globulinen liegt, während die Hexosewerte der beiden anderen Paraproteine denen der normalen Plasmaproteinfraktionen entsprechen. Es wäre aber auch möglich, dass diese Unterschiede auf den von Horejší und Smetana (3) selbst beschriebenen Einfluss unterschiedlicher Rivanolchargen auf den Fraktionierungsvorgang zurückzuführen sind, obwohl wir selbst bei Anwendung eines acht Jahre gelagerten Präparates und eines völlig frischen anderer Herkunft keinerlei Unterschied feststellen konnten.

In einer ausführlichen Studie untersuchten Šťastný und Hořejší (11) die physikalisch-chemischen Bedingungen bei der Interaktion von Acridinfarbstoffen mit Plasmaproteinen. Die dabei erzielten Daten betreffend den Einfluss von pH und Ionenstärke kongruieren mit unseren Ergebnissen. Boettcher et al. (1) finden bei der Arbeit in pH-Bereichen unter 8,0 — über die vorliegenden Ionenstärke machten sie keine Angaben — ebenso wie auch andere Autoren Albumin in der Rivanolfraktion. Wir meinen in Übereinstimmung mit Stastny und Horejší (11), dass der Albumingehalt dabei in erster Linie eine Folge der jeweiligen Ionenstärke ist.

Die Fraktionierung von Plasmaproteinen mit Hilfe von Rivanol stellt eine wertvolle Bereicherung der eiweisschemischen Methoden dar, die auch für den Kliniker von Bedeutung ist, da sie sich zur raschen, orientierenden Bestimmung des Gammaglobulinspiegels im Blut in ebensogutem Masse eignet wie Kunkels Turbiditätstest mit Zinksulfat (4), für den biochemisch arbeitenden Mediziner ist sie sehr gut brauchbar als ein Schritt im Laufe der Auftrennung des Blutserums. Auch in Verbindung mit Ionenaustauschern hat Rivanol zur Darstellung von Thrombinen Anwendung gefunden (9).

Summary

Investigations, particularly by means of immunoelectrophoresis, agar gel precipitation and analytical ultracentrifugation, revealed a high content of γ_1-globulin, γ_{1A}-globulin, transferrin and haemopexin in the blood plasma fraction obtained by adding a 0.4 per cent aqueous solution of Rivanol to blood serum in the relation 1 : 3.5. This fraction was nearly independent of the pH and the ionic strength while the solubility of the other plasma protein fractions was dependent on these two factors. The γ_1-macroglobulin was regularly precipitated and the rheumatoid factor, as was to be expected, was not contained in the Rivanolfraction.

Dem schwedischen Institut für kulturellen Austausch mit dem Auslande und dem Prof. Nanna Svartz Fond bin ich für finanzielle Unterstützung zu Dank verpflichtet.

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From The Medical Department (Head Kaj Kjerulf) and the Dental Clinical (Head E Hjorting Hansen), Blegdams Hospital Copenhagen Denmark

Technique in Repeated Tongue Biopsies

By

E Hjorting Hansen, Henning Jensen and Kaj Kjerulf

There is still a need for evaluation of the histo pathological structure of the tongue in patients suffering from various general medical diseases So far most investiga tions in this field have been based upon single biopsies The ideal tongue biopsy would be a non painful procedure which could be repeated even several times thus permitting the individual patient to be his own control The anaesthesia should not modify the tissue used for biopsy

Taft *et al* in 1958 (3) presented an article based on 50 tongue biopsies performed with a cutting instrument similar to that generally used for ventricle mucosal biopsy Epithelium with papillae and the underlying con nective tissue but not the musculature of the tongue was sucked into the opening of the knife This procedure however might fail with atrophic and rigid tongues as happened in 3 of 33 biopsies Also the infiltration analgesia employ ed in this series might interfere with the histological picture

A technique similar to that described in this paper except for infiltration analgesia of the biopsy area has been employed by Silverman *et al* (2) in leucoplakias of the buccal mucosa

Methods

Analgesia A tentative biopsy performed under local surface analgesia with xylocain adrenalin sprayed directly on the area was very uncomfortable for the patient and the tissue sample was oedematous Infiltration analgesia around the biopsy area was like wise too painful and again the tissue sample was sometimes oedematous

Finally block analgesia of the lingual nerve was adopted Xylocain (2 %) adrenalin solu tion was injected close to the lingual nerve peripheral to the inferior alveolar nerve The injection should be made about 1 cm over the occlusal surface of the inferior last molar tooth and on the internal side of the frontal rim of the vertical mandibular bone (crista temporalis) Should the patient have lost his teeth the injection is made just between the posterior parts of the toothless inferior and superior alveolar processes The needle having been brought into contact with the

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Fig 1 Lingual nerve block analgesia in tongue biopsy



Fig 2 Incision in tongue biopsy

bone is withdrawn about 1 mm before the injection is made (fig 1) (Lingual nerve block analgesia in tongue biopsy) Only the lingual nerve is analgesized not the inferior alveolar nerve as in other surgical or dental procedures involving this area (1) In tongue biopsies this analgesia comprising the lingual nerve i e the anterior 2 3 of the one side of the tongue and the corresponding part of the bottom of the oral cavity has been found fully sufficient

Patients describe the analgesia as having a duration of about 2 hours

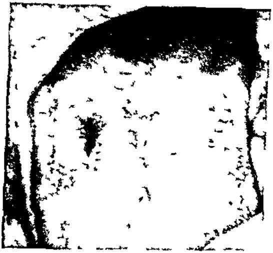


Fig 3 Tongue biopsy lesion 15 minutes after the cutting

Incision In order to avoid major arteries and to obtain a suitable number of papillae in the sample all biopsies were made in the same area i e midway between the margo lateralis sulcus medianus linea terminalis and apex of the tongue Use was made of a cylindrical cutting instrument (fig 2) (Incision in tongue biopsy, originally made for skin biopsies) The inner diameter in this study was 5 mm, which give a cylinder of tissue large enough for further division into samples for different purposes — light microscopy, electron microscopy, and eventual histochemical examinations The tissue sample when cut is pinced and made free by a tiny pair of scissors from the child surgery service The edge of the cutting instrument should be very sharp and only used once before resharpening

Haemostasis is very simply achieved with a gauze swab which the patient has to press between the bleeding part of the tongue and the palate In persons without a bleeding tendency haemostasis is obtained within ca 5 minutes The gauze swab should however even then be kept in place for the following 15 minutes In view of the rather big tissue sample removed the lesion will very soon seem small owing to the immediate contraction (fig 3) (Tongue biopsy lesion 15 minutes after the cutting) Already after 2 days healing is usually complete The wound

undergoes *no significant* reaction due to infection and no haematoma of the tongue has so far been observed in more than 80 biopsies performed

Contra indications are first of all increased bleeding tendency Every biopsy performed should be preceded by a determination of at least bleeding time, blood coagulation time number of thrombocytes and prothrombin level which should be normal or clo e to normal values, i e the same criteria as used in needle biopsy of kidney or liver

The authors have no experience so far with tongue biopsies in patients with acute infectious diseases, acute infection of the mouth cavity, however, has been considered to be a contra indication

Complications Of 80 biopsies performed 78 were without complication In 2 patients the criteria for normal bleeding time coagulation time, number of thrombocytes and prothrombin level were not fulfilled and subsequently complications occurred as follows

1 A 75-year-old man with fully developed untreated pernicious anaemia had a tongue biopsy in spite of bleeding time 8 5 minutes blood coagulation time 6 5 minutes thrombocytes 25 000/μl and prothrombin concentration 58 % of normal level After the routine gauze compression haemostasis was apparently complete in 10 min and no suture was applied Two hours later the biopsy lesion started a small constant bleeding which increased gradually over the following 4 hours whereafter the wound was closed with two tiny silk sutures and a subsequent complete and immediate haemostasis was obtained with no further complications When the biopsy had been performed treatment was initiated with vitamin B_{12} injections and 14 *days later* a second biopsy was performed this time without any precaution (suture) and without any bleeding complications The bleeding and coagulation times had improved to 4 5 and 5 5 minutes respectively and the thrombocyte number had increased to 148 000 the prothrombin level was found unaltered at 56 % of normal value

2 A 68-year-old man who had been bedridden for the last 6 months on account of verified advanced multiple myelomatosis with several fractures of the lumbar spine His bleeding time was prolonged 13 minutes coagulation time 4 5 min thrombocytes 152 000/μl and prothrombin level 49 % of normal values Sternal marrow biopsy was repeated without bleeding complications and a tongue biopsy was ventured in spite of the above mentioned abnormal bleeding conditions The biopsy lesion was closed with 3 silk sutures and after gauze compression of the tongue for 10 minutes haemostasis was sufficient and remained so for the following 12 hours Hereafter the patient gradually developed constant and severe bleeding from the biopsy wound and owing to his preceding poor condition he developed bleeding shock The condition proved refractory to intravenous vitamin K injections and 3 transfusions of fresh donor blood and hydrocortisol intravenously had to be administered to stop the bleeding definitely whereafter the biopsy wound healed as usual

A third patient had a significantly *prolonged* bleeding time and bleeding after a tongue biopsy had to be foreseen The biopsy lesion therefore was sutured immediately after the cutting and there was in fact *no bleeding* probably as a consequence of the prophylactic suture

Summary

The technique is described for tongue biopsy involving the mucosa and some of the underlying muscular tissue of the

tongue, by means of a punch technique with block analgesia of the lingual nerve. So far 80 biopsies have been performed. Seventy-eight of these were completely uncomplicated. Prolonged bleeding, however, occurred in 2 cases where the criteria for normal bleeding conditions were not fulfilled.

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From the Hospital for Infectious Diseases Uppsala Sweden

An Extensive Outbreak of Gastroenteritis Caused by Salmonella Newport

II Some Clinical Observations on 488 Hospitalized Cases and the Results of Cultures

By

TORE MELLBIN, FOLKE NORDBRING and BO BILLE

An outbreak of *Salmonella Newport* food poisoning occurred in Uppsala at the end of April 1960 The source of infection was butter cream used in different types of pastry The course of the epidemic has been described in the first paper of the series (1)

Altogether 745 infected cases were recorded The distribution of different grades of severity according to given definitions (1) among 626 patients from Uppsala itself was as follows 1 1 % were dangerously ill of whom one patient died of salmonella sepsis 24 8 % had an illness of moderate severity 8 3 % had a very mild illness without fever and no less than 65 8 % had no symptoms at all but were carriers

The incubation period was less than 48 hours in the majority of the cases but 28 % had an incubation period of 3—7 days and 4 % one of 8—12 days (1)

The period to freedom from bacilli in faeces was remarkably long Only 50 % of the patients were free of bacilli after six weeks of illness As many as 15 % were still excreters after nine weeks (1)

The present paper deals with the symptomatology of the 488 patients admitted to the Uppsala Hospital for Infectious Diseases The results of blood and urinary cultures are reported

Several antibiotics and other drugs were used mostly with the purpose of shortening the period of excretion of bacteria in the faeces The results of these trials are discussed

The results of serological studies on 412 patients have been presented in the third article of the series (6)

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TABLE I. Distribution according to age, sex and different grades of severity of illness of 488 hospitalized patients

Age group (years)	Grades of severity of illness: Severe ♂	♀	Moderate ♂	♀	Slight ♂	♀	Carriers ♂	♀	Total ♂	♀	%
<1				2		1	1	4	1	7	1.6
1–6			12	5	2	2	12	15	26	22	9.8
7–15		1	5	10	3	2	52	37	60	50	22.6
16–24			12	15	4	9	19	37	35	61	19.7
25–59	2	3	24	30	12	7	54	53	92	93	37.9
≥60	3		9	10	1	4	6	8	19	22	8.4
Total	5	4	62	72	22	25	144	154	233	255	100.0
	9		134		47		298		488		
%	1.8		27.5		9.6		61.1		100.0		

Pertinent references in the literature concerning various problems in connexion with salmonella infections have been given in the first and third papers of the series (1, 6).

Material and methods

Of the 488 patients admitted 416 lived in Uppsala and 72 in the surrounding country.

The distribution according to age, sex and grades of severity of illness of the hospital material is given in table I. The proportions are almost identical with those of the total Uppsala material (1).

Faecal specimens were cultured regularly, usually every third day.

Blood and urinary specimens for culture were obtained from a reduced material of 380 patients, because 108 patients isolated in a provisional hospital building outside the town had to be excluded for technical reasons. The distribution of patients with different grades of severity of illness within the reduced material was 2.4 % with severe symptoms, 32.6 % with moderately severe symptoms, 10.5 % with mild symptoms and 54.5 % were carriers.

No catheterization was carried out. It was not possible to make quantitative urinary cultures.

All specimens were cultured in the Bacteriological Laboratory, University of Uppsala.

Symptomatology

Data were available from 142 patients with a severe or moderate illness and from 47 patients with a mild illness without fever.

Fever. High fever for a number of days with moderate remissions similar to the pyrexia of paratyphoid fever was common. In some instances the fever was continuous with only slight remissions suggestive of the fever curve of typhoid fever. Most frequently the fever arose suddenly, not seldom combined with rigors, abating gradually in the course of a few days. Three patients had high fever but no diarrhoea. On an average every third patient developed a pyrexia of 40° C or more and every third adult experienced rigors.

The duration of the fever was known in 133 instances. On an average the fever

lasted for seven days in children (range 2—14 days) for six days in adults of the age of 16—59 years (range 1—15 days) and for ten days in adults of the age of 60—81 years (range 2—25 days) Thus elderly people showed a tendency to be febrile for a protracted time Prolongation of the period of fever owing to complications such as pneumonia was not taken into account

Diarrhoea The great majority of the patients with severe or moderate illness suffered from diarrhoea of extreme intensity and frequency Some were in great misery, completely incapable of controlling the bowel movements Visible blood in the stools was not typical

The duration of the period of diarrhoea was known in 175 instances The average duration for the febrile patient of any age was seven days (range 1—15 days) for the afebrile patient three days (range 1—9 days)

Other symptoms Approximately half of the febrile patients vomited one third experienced abdominal cramps and a quarter suffered from headaches Many were markedly exhausted at the onset of the symptoms The fever recurred in eight instances Splenomegaly was noticed only twice rose spots only once

Three case histories will be reported briefly to exemplify the septic and serious course of disease which may result from infection with *S Newport*

Case No 101 A housewife 28-year-old previously healthy Mode of infection was unknown The patient developed high fever fig 1 backache and intense diarrhoea She became extremely exhausted and fainted On admission she was prostrated and dehydrated with proteinuria and increased NPN Marked improvement was attained by intravenous fluids but the fever lasted for 12 days *S Newport* was isolated from the blood and the faeces An O V III agglutinin titre of 1/80 was found on the eighth day of illness The patient excreted *S Newport* for seven weeks

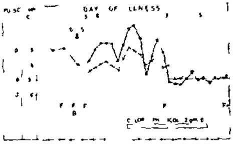


Fig 1 Temperature chart of case No 101 ●—● denotes the temperature ○ ○ the pulse rate F is faecal culture B blood culture

Comment

The case demonstrates the serious effects of a salmonella septicaemia on even a young, healthy adult

Case No 155 A man 76-year-old hitherto in excellent health He was taken ill with vomiting and diarrhoea of marked intensity 24 hours after the ingestion of infected cakes The temperature was not known On the eighth day he was found on the floor, unable to get up and was brought to the hospital The patient was afebrile but weak and dehydrated There was a moderate electrolyte imbalance elevated Hb and NPN values and proteinuria with a normal urinary sediment Faecal urinary and blood cultures were all positive He received fluids intravenously and a blood transfusion but developed cardiac failure with pulmonary oedema and died on the eleventh day of illness

On autopsy sepsis was revealed with heavy growth of *S Newport* in the following organs intestines blood gallbladder heart spleen and kidneys

Comment

Salmonella septicaemia may be particularly dangerous in elderly people as the case illustrates Many other old patients of the epidemic were gravely ill, but no other deaths occurred

Case No 191 An assistant nurse 53 year-old, previously healthy Two days after consumption of infected cakes she experienced rigors and suffered from moderate fever and mild diarrhoea for three days On the eighth day she felt severe pain in the region of the *symphysis pubis* and was unable to walk During the next few days remittent fever appeared and the pain was aggravated The patient was admitted on the eighteenth day Faecal cultures and a blood culture revealed growth of *S Newport* The O VIII agglutinin titre was 320 The LSR was elevated The symptoms subsided after chloramphenicol treatment, but she continued to excrete organisms for seven weeks Radiological examination of the skeleton two months after the onset of symptoms revealed osteitis in the pelvic bones

Comment

In all probability the osteomyelitis found was caused by *S Newport* The first symptoms were of the moderate severity very commonly observed in our patients Yet penetration of the salmonella organisms into the blood stream occurred, with a subsequent focal manifestation in the skeleton

Cultures

Blood cultures Blood specimens for culture were obtained from 26 febrile patients with a severe or moderately severe illness Growth of *S Newport* was revealed in no less than 15 instances (57 7 %) The positive blood specimens were withdrawn on the 3rd to 7th days of illness in 12 cases and on the 11th 17th and 19th days in three complicated cases

Urinary cultures Samples of urine for culture were obtained from 350 patients (92 1 % of the material) Growth of *S Newport* was demonstrated in 14 2 % of 120 febrile patients in 8 1 % of 37 patients with mild illness without fever, while only 3 out of 193 carriers (1 6 %) were positive Altogether, 23 patients (6 6 %) had positive urinary cultures 11 of whom were adults.

A considerable difference in results between the sexes was noted Many more females particularly young girls than males showed growth of *S Newport* in the urine Half of the girls with symptoms of disease had positive urinary cultures, a frequency which did not correspond well to that found in the adult woman The only carriers with positive urinary cultures were children Besides the fact that women more readily contract an ascending infection of the urinary tract than men the divergence between the sexes must give rise to the suspicion that contamination of the urine specimen at the moment of sampling from the female patient was unavoidable to a certain extent

However, the frequency of positive urinary cultures among adult men and women with a febrile illness did not differ appreciably (8 5 and 12 0 %, respectively) Contamination seems to have played a minor role in this age group Blood cultures were made in six of the adult cases with growth of *S Newport* in the urine all being positive None of the 120 adult carriers had a positive urinary culture

Examination of the urinary sediments of the patients with positive urinary cultures revealed pyuria in the samples from all adult women while a few adult men had normal sediments, even those with a positive blood culture

Growth of *S Newport* in the urine was for the most part found during the first four weeks of illness All urinary cultures were negative after the sixth week

Results of therapeutic trials

Chloramphenicol This antibiotic was administered to only a few febrile patients in the acute stage of the disease The total dose given was maximally 24 g The effect on temperature and general condition was excellent, but as expected the drug did not influence the excretion of bacilli, except temporarily (table II)

TABLE II Results of different therapeutic trials

	Patients treated				
	Total	Negative faecal cultures in connexion with course		Permanent effect	
	No	No	%	No	%
Chloramphenicol	11	3	(27.3)	0	—
Ph 778	45	22	48.9	14	31.1
Enterobiotic + Felamin	33	33	100.0	2	6.1
Terramycin + Meticorten followed by Enterobiotic + Felamin	11	11	100.0	5	(45.5)
Humatin	6	6	100.0	1	(16.7)
All types	106	75	70.8	22	20.8
Placebo	55	16	29.1	6	10.9
Felamin	28	9	32.1	3	10.7

Ph 778 (Pharmacia) This substance, 2 (5 nitro 2 furyl) 5 amino 1 3 4 thiadiazol which belongs to the *nitrofuran* group of antibacterial agents has been shown to have a marked inhibitory *effect* on different salmonella strains in the laboratory (9) The drug was administered by mouth in a dose of 200 mg three times a day for 10 days All *courses* of treatment took place during the first three weeks of infection Nausea was a not uncommon side effect

The results of the treatment with Ph 778 should be compared with the results of the placebo group (table II) For a period every other *patient* was *given by* chance either Ph 778 or a *placebo* preparation in the dose stated *The two* groups are quite *compatible* with regard to age and sex distribution 57.8 % of the patients in the Ph 778 group had *severe or* moderate symptoms and 58.2 % of the patients in the *placebo* group had an illness *of corresponding* severity

It will be seen from the table that *half of* the *patients* given Ph 778 had negative faecal cultures in immediate connexion with the course but the *effect* was permanent in only *one* third It should be noted that one third of the patients *in the placebo* group *had spontaneously* become *free of bacilli during* the course and one tenth were *definitely free*

Enterobiotic® *(Pfizer)* + *Felamin*® *(Sandoz)* This antibiotic combination (neomycin + terramycin) was administered in a dose of four tablets four times daily *for* two days together with a choleretic (Felamin) *one* tablet three times daily *The* aim was to empty the gallbladder *and sterilize* the intestinal content The treatment was instituted in long standing carriers

The *immediate* effect was as intended, with complete sterilization of the faeces

TABLE III Cumulative percentages of treated and untreated patients free of bacilli within different numbers of weeks All courses of treatment took place within the first 3 weeks of infection Febrile patients had a disease with severe or moderately severe symptoms afebrile patients displayed mild symptoms or were carriers Patients for whom the day of contraction of the infection is unknown are excluded

Free from bacilli within (weeks)	All patients				Patients treated with			
	Treated		Untreated		Ph 778		Placebo	
	Febrile n=38 %	Afebrile n=28 %	Febrile n=98 %	Afebrile n=170 %	Febrile n=26 %	Afebrile n=19 %	Febrile n=30 %	Afebrile n=22 %
3	10 5	35 7	4 1	22 4	15 4	52 6	0	36 4
6	55 3	75 0	33 7	51 8	69 2	100 0	36 7	72 7
9	81 6	92 9	71 4	87 6	88 5		76 7	90 9
12	89 5	96 4	89 8	99 4	92 3		83 3	100 0
15	97 4	100 0	94 9	99 4	96 2		96 7	

for a few days without any ill effects worth mentioning However, the long-term effect was quite disappointing (table II)

Felamin only was given to a "control" group of 28 patients The effect was compatible with that of the placebo (table II) and, as concerns permanent freedom from bacilli, as good as when enterobiotic was administered simultaneously

Terramycin® *(Pfizer) + Meticorten*® *(Schering Corp) followed by Enterobiotic + Felamin* This combination of prednisone and antibiotics was tried in some very persistent carriers for the purpose of strengthening the antibiotic effect The doses were terramycin 2 g daily for five days, meticorten 40—20 mg daily for five days, enterobiotic + felamin as previously

The intestinal contents became sterile in all instances (table II), but half of the patients treated continued to excrete bacilli later on

Humatin® *(Parke-Davis)* This antibiotic, paromomycin, has been shown to produce a rapid decrease in the number of organisms in the intestines after oral ingestion (8), and the drug has been used in the treatment of salmonella infections with some success (2 4, 5) Humatin was given to a few persistent carriers in a large dose (5g daily in divided dosage for five days) The immediate effect was good in 100 % but the lasting effect poor (table II)

In summary, 70 % of the 106 patients receiving various antibiotics had negative faecal cultures during the course of treatment or immediately thereafter, but only 20 % were permanently free of bacilli, a result not particularly encouraging

However, if we consider only the patients treated early, during the first

three weeks of illness, and compare the time elapsing to definite freedom from bacilli with that of the untreated patients, a certain effect seems to have been gained This is shown in table III Throughout, the patients treated became free of bacilli faster than the untreated ones, but the effect can be observed only for nine weeks Particularly Ph 778 seems to have reduced the length of excretion Antibiotic treatment of other kinds, instituted later, was equal in the Ph 778 and placebo groups and the effect of the subsequent treatment was equally poor in both groups

Discussion

The clinical observations of acutely ill patients with a *S Newport* infection in the present epidemic have revealed that a *septic state* with high fever a condition similar to paratyphoid fever, is more *common than* generally thought True septicaemia could be proved in 58 % of the patients cultured Urinary infection occurred in approximately 10 % of the adults In the majority of the adult cases the bacterial growth observed in the urine should probably be interpreted as a focal manifestation of a septicaemia because several patients had a positive blood culture Osteomyelitis was observed once

Although gastrointestinal symptoms usually predominate in salmonella food poisoning as was the case in our material it is thus advisable to suspect a bacteraemia and make blood cultures Urinary cultures should also be made From a public health point of view the patient excreting salmonella organisms in the urine must be considered more dangerous than the faecal excreter, because his attention is focused on the danger of faecal contamination, and he is not aware of the risk emanating from the urine

The problem of therapy for the salmonella carrier state is unsolved Antibiotics such as chloramphenicol are of great value in the treatment of the acute septic condition, but no reliable method of shortening the period of excretion is yet known (3, 7) On the whole, our data support this opinion Ampicillin was not available at the time of the epidemic, but our later experiences in other cases of salmonella enteritis are not convincing

The physician is in the delicate situation, when starting a course of treatment, that he is not able to promise a permanent effect, and sampling of faecal specimens for reliable cultures must be postponed until some days after termination of the treatment Some patients might have been spontaneously free of bacilli independently of treatment (this occurred in 10 % of the patients of the placebo groups), and the period of isolation may thus be unnecessarily prolonged because of the treatment It is also disappointing to be compelled to note that the patient has resumed excretion of the organisms after a course of treatment, seemingly successful from the beginning but actually a failure

Yet our data seem to entitle us to conclude that a treatment instituted early in the course of the disease may reduce the length of the carrier state Particularly the nitrofuran substance seems to have had such an effect How-

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3	10.5	35.7	4.1	22.4	15.4	52.6	0	36.4
6	55.3	75.0	33.7	51.8	69.2	100.0	36.7	72.7
9	81.6	92.9	71.4	87.6	88.5		76.7	90.9
12	89.5	96.4	89.8	99.4	92.3		83.3	100.0
15	97.4	100.0	94.9	99.4	96.2		96.7	

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ever, the other antibiotics used were mainly given to longstanding, stubborn cases Possibly we would have arrived at the same result with antibiotics sterilizing the intestinal content, had they been administered earlier

Summary

Clinical data concerning 488 hospitalized cases of infection with *Salmonella Newport* are presented *1 8 % had a severe,* dangerous disease, 27 5 % had a febrile illness of moderate severity, 9 6 % had but slight symptoms, and 61 1 % were carriers only

The patients with a severe or moderate illness commonly exhibited a septic type of fever, similar to the pyrexia of paratyphoid fever Blood cultures revealed growth of *S Newport* in 58 % of the patients investigated Urinary cultures were positive in approximately 10 % of the adult patients with fever, while none of the adult carriers showed growth of *S Newport* in the urine There was one case of probable salmonella osteomyelitis One death occurred from salmonella sepsis

Infection with S Newport may thus result in not only a more or less innocuous gastroenteritis but often in a true sepsis, which might be hazardous

The results of therapeutic trials with several antibiotics were rather disappointing An immediate effect in direct connexion with the course was secured in 70 % of the cases, but the effect was permanent in only 20 % A certain result of therapy was found, however, implying a reduction of the length of the carrier state in cases which received *treatment early in the course of the* disease

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Experiences with ε-Aminocaproic Acid (ε-ACA) in the Treatment of Profuse Menstruation

By

Inga Marie Nilsson and Sven Erik Björkman

The incidence of profuse menstrual bleeding in women without any demonstrable gynaecological disease is unknown Chronically increased menstrual blood loss is generally recognized as an important cause of iron deficiency anaemia

The aetiology of increased menstrual flow is not known Hormonal dysfunction and iron deficiency as such have been discussed as a possible explanation

Albrechtsen (2) found human endometrium to contain a plasminogen activator The concentration of this activator varied with the phases of the menstrual cycle It was highest in the secretory stage He assumed that the tissue activator of the endometrium was of significance in the occurrence of uterine bleeding He also found an increase of this tissue activator in the endometrium in women with proliferative bleeding The senile endometrium did not contain tissue activator Similar findings have since been reported by Kullander and Kallén (13) and Fuhrmann (10)

ε ACA, which is an inhibitor of plasminogen activation, has proved useful in bleeding caused by fibrinolysis (1 4, 5, 7, 8, 9, 11, 14, 15, 16, 20 22)

Nilsson et al (16) administered ε-ACA not only to patients with proven fibrinolytic bleedings but also to 10 patients with bleeding symptoms but without any demonstrable fibrinolytic activity in the circulating blood These patients suffered from profuse menstrual bleeding, chronic ulcerative colitis with rectal haemorrhage or polycystic kidney disease with haematuria The bleeding stopped This effect was ascribed to inhibition of a local action of fibrinolytic activators It has for example, been found that administration of ε ACA stops bleeding in the urinary tract, and that this effect is due to inhibition of the local action of urokinase (6 7, 8 14, 22)

The Japanese workers (23) reported ε ACA to be effective in the treatment

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TABLE I Survey of clinical data

Case	Age (years)	Married or single	No of children	Duration of profuse menstrual flow (years)	Gynaecologic examination	Anaemia Duration (years)	Unheated fibrin plates lysed area (mm²) (citrated plasma)	Fibrinogen (g/100 ml)	Other coagulation factors
1	15	S	0	2	Normal	2	39	0 38	Normal
2	18	S	0	5	Normal	5	0	0 40	Normal
3	21	S	0	7	Normal	7	0—50	0 34	Normal
4	23	S	0	9	Normal	9	0—54	0 25	Normal
5	27	M	0	11	Normal	11	0—20	0 26	Normal
6	27	M	0	14	Normal	14	0—51	0 31	Normal
7	28	M	2	16 (sporadically)	Normal	16	0	0 26	Normal
8	28	M	0	15	Normal	15	0	0 29	Normal
9	30	M	3	½	Normal	½	0	0 33	Normal
10	30	M	3	16	Normal	16	34	0 30	Normal
11	33	M	4	>10	Normal	15	0	0 38	Normal
12	37	M	2	>10	Normal	10	32	0 24	Normal
13	35	M	1	20	Normal	20	0—57	0 28	Normal
14	21	S	0	7	Normal	7	0	0 27	Normal
15	42	M	2	10 (sporadically)	Normal	10	37	0 25	Normal
16	42	M	3	6	Normal	6	0	0 29	Normal
17	43	M	1	5—6	Normal	5-6	45	0 25	Normal
18	47	M	0	5	Normal	5	0	0 40	Normal

of metropathia haemorrhagica The fact that ε-ACA can inhibit the local action of tissue activators prompted us to study whether administration of ε-ACA might reduce the fibrinolytic activity of the endometrium and thereby decrease the haemorrhage

Since 1960 we have treated 24 patients without any gynaecological disorder but with profuse menstrual flow Eighteen of these patients were otherwise healthy women and 6 suffered from a haemorrhagic diathesis The results are presented below

Material and methods

The clinical material consisted of 24 women with profuse menstrual flow Six of these patients suffered from a haemorrhagic diathesis The other 18 had no demonstrable coagulation abnormality

All patients were examined gynaecologically and in the majority of cases curettage was performed Blood counts were *done before and during treatment* Coagulation analyses including all the tests given under methods" were performed in all *cases*

Clinical data are summarized in tables I—III Cases 1, 4, 8, 9 and 10 have been reported previously (*16*)

TABLE II Survey of clinical data Cf detailed case reports of cases 5 6 13 17 and 18

Case	Age (yrs)	Weight (kg)	Total dose/menstrual period (g)	No of periods treated	Total amount of ε ACA given (g)	Effect on menstrual flow	Hb (g/100 ml) Before treatment	After treatment	Side effects
1	15	60	12–30	6	150	Normalized	10 2	13	None
2	18	57	18	1	18	Normalized	12 0	—	Nausea
3	21	64	12–24	2	36	Normalized	10 4	–	Nausea Dizziness
4	23	48	15–25	6	105	Normalized	11 2	13	Dizziness
5	23	56	10–30	19	340	Normalized	10 4	13 3	Dizziness Nausea
6	27	62	30–48	4	174	Normalized	6 8	11 2	[1]None
7	28	66	25	1	25	Normalized	11 0	—	Nausea
8	28	62	18	3	54	Normalized	10 0	13 0	[1]Nausea
9	30	70	28 66	3	122	Normalized	10 2	12 3	Nausea
10	30	73	28–56	2	84	Normalized	10 0	12 0	Nausea Dizziness
11	33	65	24	1	24	Normalized	—	—	Nausea
12	37	62	30–48	3	114	Normalized	11 0	12 5	Nausea Dizziness Diarrhoea
13	35	58	12–48	7	224	Normalized	9 6	12 2	None
14	21	51	20 25	3	70	Normalized	11 7	—	Nausea
15	42	58	6	1	6	Decreased	11 0	13 0	Nausea
16	42	70	77	1	77	Normalized	—	–	None
17	43	62	24 90	5	294	Normalized	10 2	12 8	Dizziness Nausea
18	47	63	12 36	9	228	Normalized	7 4	14 0	None

[1] Pregnant during treatment

ε-aminocaproic acid (ε ACA) A 20 % syrup of ε ACA was used for administration by mouth (Epsikapron[R] supplied by AB Kabi Stockholm Doses of 1 g 10 kg body weight were given at various intervals

Coagulation studies The following determinations were made coagulation time bleeding time platelet count one stage prothrombin time prothrombin + factor VII factor V AHF (f VIII) haemophilia B-factor f IX plasminogen - proactivator serum inhibitors of plasminogen activation and various tests for fibrinolytic activity The methods for collection and preparation of blood and for measuring the different coagulation factors and components of the fibrinolytic system have been described earlier (17 18 19 21)

Results

Profuse menstruation and normal coagulation mechanism

The effect of ε ACA on 18 healthy women is summarized in tables I and II Seven (2 3 10 11 12 14 and 17) of

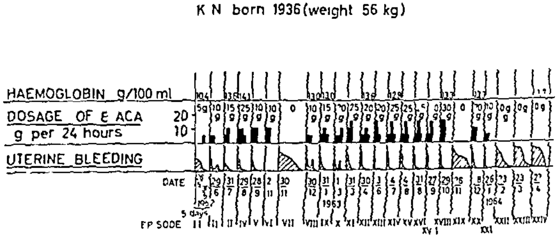


Fig 1 The course in case 5

them did not continue to take the drug because they could not tolerate its side effects In cases 7 and 15 the menstrual flow varied considerably in amount from one period to another and treatment was discontinued In cases 5, 10, 13, 14 and 17 ε ACA was withdrawn for one to three cycles during the treatment and then bleeding was profuse In cases 1, 4 and 9 therapy was continued for 6, 6 and 3 periods, respectively, after which menstruation became, and remained, normal

Detailed case reports of cases 5, 6, 13, 17 and 18 are given below

Case 5, born 1936 A 27 year old nulliparous married woman had since the menarche had profuse menstrual bleeding lasting for 7 to 10 days Owing to these haemorrhages she was anaemic with haemoglobin values of about 10 g/100 ml Gynaecological examinations on several occasions failed to reveal any cause Curettage had no lasting effect Coagulation analyses showed normal values throughout No fibrinolytic activity could be demonstrated in the blood Since May 1962, in 19 menstrual periods, she has been treated with ε ACA in oral doses of 5 g (fig 1) She has taken between 10 and 30 g during the menstrual period on the first to third day The bleeding decreased considerably and did not last longer than 4 days During this time she had haemoglobin values ranging between 12 8 and 14 1 g/100 ml She was, however troubled by dizziness and nausea During period VII and XIX no ε ACA was given The bleeding was then profuse and prolonged In Feb 1964 we stopped the therapy The menstrual flow again increased and she became again anaemic

Case 6, born 1935 A 27 year-old married woman She had no children Since the age of 20 she had been troubled by profuse and prolonged menstrual flow, which forced her to stay in bed for about one week every month She was always anaemic The haemoglobin values ranged between 6 5 and 9 g/100 ml despite continuous iron therapy Gynaecological examinations revealed no cause Curettage had been performed on several occasions but without effect The bleeding was so profuse that the doctors at her hospital suspected a haemorrhagic diathesis and referred her to us for coagulation analysis Coagulation studies showed normal values throughout On admission the haemoglobin value was 6 8 g/100 ml and RBC 2 8 mill /mm³ She was treated with ε ACA during 4 menstrual periods

Period I The patient bled profusely on the first day The haemoglobin value was 6 8 g/100 ml On the second day the patient

received a dose of 6 g of ε ACA by mouth The bleeding decreased and the effect lasted for about 5 hours She was then given a second dose of 6 g of ε ACA The bleeding again decreased and the effect lasted for about 6 hours She was then given a further dose of 6 g of ε ACA and this time the effect lasted for about 13 hours On the third day the patient received one dose of 6 g of ε ACA and the bleeding remained normal the whole day On the fourth day the bleeding again increased but almost disappeared after 6 g of ε ACA She was not troubled by dizziness or nausea

Period II On the second day of menstruation the patient received 6 g of ε ACA with good effect *The next day medication was* skipped and the patient bled profusely On the fourth day the patient was given 3 doses of 6 g of ε ACA at 6 hour intervals and the bleeding decreased On each of the following two days the patient had two doses of 6 g of ε ACA The bleeding ceased The haemoglobin value had increased to 9 2 g 100 ml

Period III and IV The flow was initially profuse On the first day the patient received 3 doses of ε ACA on the second and third la   doses and on the fourth day one dose of 6 g of ε ACA The bleeding was not   of se The haemoglobin value was 11 2 g 100 ml The patient was feeling well She had   ed to take iron

The   ext month menstruation did not appea   Examination revealed that the   was pregnant In Sept 1963 she   a normally developed girl   pregna   and the parturition were   al   When menstruation returned was no longer profuse

*Case   born 19   * A 35-year-old married   had since the menarche had profuse   al bleeding lasting for 7 to 10 days She had   child born 1957 Owing to these bleedings she had always been anaemic   haemoglobin values ranging between 8 and 10 g 100 ml despite iron therapy Gynaecological examinations on several occasions revealed no cause Curettage had

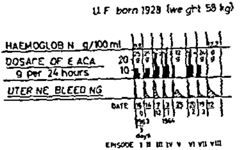


Fig 2 The course in case 13

no lasting effect Investigation of coagulation factors revealed no abnormality The blood did not show any fibrinolytic activity

Period I The patient received 2 doses of 6 g of ε ACA on the first and on the second day of menstruation On the third day the patient was given one dose of 5 g of ε ACA The bleeding was not profuse and did not last for more than 4 days (fig 2)

Period II An initially profuse menstruation During the first 3 days the patient had 5 doses of 5 g of ε ACA in the same way as during period I The bleeding was not profuse

Periods III and IV ε ACA was given in doses of 6 g all together 42 and 48 g respectively in the course of 3 days On the second day of menstruation the effect of one dose lasted only about 6 hours

Period V On the first day of menstruation the patient received 2 doses of 6 g of ε ACA The bleeding decreased considerably Then no further ε ACA was given The patient again started to bleed rather heavily and the bleeding lasted for 10 days

Period VI The patient received ε ACA in the same way as during episode IV The effect on the bleeding was good

Period VII On the first day of menstruation the bleeding was controlled by 2 doses of 6 g of ε ACA Two doses were also sufficient on the second day The following day the bleeding remained normal without ε ACA

Period VIII No ε ACA was given The patient bled profusely for 8 days

TABLE III Administration of ε ACA to patients with profuse menstruation and haemorrhagic diathesis

Case	Diagnosis	Age (yrs)	Weight (kg)	Administration of ε ACA: Total dose/menstrual period (g)	No of periods treated	Total amount of ε ACA given (g)	Effect on menstrual flow	Hb (g/100 ml) Before	Hb (g/100 ml) After	Side effects
19	Cong haem diath with prolonged bleeding time	14	42–52	75–420	18	2 958	Normalized	See case report		None
20	Thrombocytopenia	17	33	6–60	12	348	Normalized	9	13	None
21	Thrombocytopenia	30	56	30–48	4	174	Normalized	11 2	12 7	Nausea
22	Thrombocytopenia (myeloblastic leukaemia)	33	60	114	1	114	Normalized	9 8	10 3	None
23	Thrombasthenia	48	50	60–180	8	1 010	Decreased	8 9	12 8	Nausea Dizziness
24	Thrombocytopenia	17	64	48–150	2	198	Normalized	4 4	15 8	None

No side effects were noted The haemoglobin value successively increased from *9 6 g/100 ml to 12 2 g/100 ml*

Case 17, born 1920 A 43 year old woman had *for 6 years been troubled by profuse* menstruation, each period lasting for about 10 days She was anaemic with haemoglobin values *of about 10 g/100 ml despite iron* therapy Gynaecological examination revealed no cause Curettage had been performed but without any lasting effect She was treated with ε ACA in oral doses of 6 g She required 3 daily doses on the first second and third day and occasionally 2 or 3 doses on the fourth day She was treated in this way during 5 menstrual periods The haemoglobin value increased from 10 2 g/100 ml to 12 8 g/100 ml Treatment was discontinued owing to severe nausea and dizziness After withdrawal of the drug the patient again bled profusely and hysterectomy *had subsequently to be performed* Histological examination of the uterus revealed nothing abnormal

Case 18, born 1915 A 47 year old woman without children She had always had a *profuse menstrual flow, which had increased* considerably during the last few years Gynaecological examination provided no *explanation* Coagulation analyses gave normal values On admission the haemoglobin value was 7 4 g/100 ml She was treated with ε ACA in oral doses of 6 g during 9 menstrual periods 12 to 30 g of ε ACA were given during each period The bleedings decreased and the haemoglobin value increased to 14 0 g/100 ml After the last episode she continued to bleed for weeks and the haemoglobin value again decreased to 10 4 g/100 ml She was admitted to the *gynaecological department and hysterectomy* was performed The uterus contained several submucous and intramurally located myomas The uterus was otherwise normal

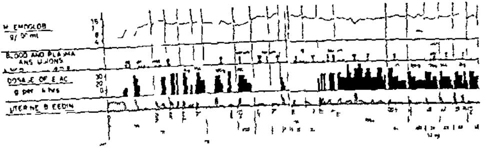


Fig. 3. Administration of ε ACA to case 19 (R. L.) with profuse menstruation and haemorrhagic diathesis

Profuse menstruation in haemorrhagic diathesis

Table III summarizes the effect of ε ACA on 6 women with profuse menstruation due to haemorrhagic diathesis. Case reports are given below.

Case 19, born 1950. This patient had a hitherto unrecognized inherited haemorrhagic diathesis characterized by prolonged bleeding time and lack of a stable plasma factor; this case will be reported in detail by Nilsson and Cronberg.

Since early childhood the patient had had an increased bleeding tendency with easy bruising, nose bleeding and persistent haemorrhage following trauma and tooth extraction.

She was otherwise healthy and normally developed. She had a *prolonged* Duke and Ivy *bleeding time* > 30 minutes and the prothrombin consumption was pathologically increased. The *platelet count* was *normal*. The plasma AHF factor VIII content was normal as were other coagulation factors studied. Clot retraction was only slightly retarded. The tourniquet test was positive. The adhesiveness of the platelets was normal and the platelets were also *found to* have normal activity with respect *to platelet* factors 1, 3 and 4. It was found possible to reduce the prolonged bleeding time to normal by injection of fresh or stored plasma (even 6 months old) or stored blood. Serum, red blood cells and platelet suspensions had no effect. Normalization of the bleeding *time proved* to require the use of plasma from donors belonging to the same blood group as the patient (B Rh +). These tests were performed on 28 different occasions from Aug. 1960 to April 1964 with equal results.

Period I. Her first *menstruation* started on Dec. 5, 1962. The menstrual flow was profuse. The *haemoglobin* value fell from 14 *g/100 ml to* 6 g/100 ml within a few days. At the end of the menstrual period 32 g of ε ACA was given for 3 days, after which the bleeding stopped. She was also given 4 *blood* transfusions but one had to be interrupted due to a severe anaphylactic reaction (fig. 3).

At period II she started to bleed profusely. The haemoglobin value fell within 12 hours *from* 12.2 *to* 8 g/100 ml. After administration of ε ACA the bleeding ceased. She also received 3 blood transfusions and 2 plasma transfusions.

At *period III* she began to bleed profusely and immediately received 2 blood transfusions and 2 plasma transfusions. The bleeding time became normal and the bleeding stopped. After withdrawal *of* this therapy she again started *to* bleed. She received 25 g ε ACA per day for 3 days and the bleeding again stopped.

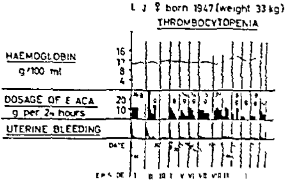


Fig 4 Administration of ε ACA to case 20 (L J) with profuse menstruation and thrombocytopenia

At *period II* the patient received one plasma transfusion and then ε ACA for 3 days The bleeding was controlled On the fifth day ε-ACA was withdrawn and she then started to bleed again After ε ACA the bleeding again stopped

At *period I* she also started to bleed when ε ACA was withdrawn after 3 days and a further dose had to be given During the following three periods it proved necessary to continue treatment with ε ACA for 7 to 11 days

In the summer months (*periods IV V and VI*) the bleeding was not profuse During *periods VII end VIII* the patient was treated with repeated transfusions of plasma which controlled the bleeding

During *period XII* treatment with ε ACA every other day was tried but proved insufficient During *periods XI and XII* she bled profusely, and the bleeding did not stop until a daily dose of 30 g of ε ACA had been given

On Dec 22 she had then not bled for 2 days, she was allowed to take a walk in the city During her walk she began to bleed profusely and went into shock Five blood transfusions were given She continued to bleed for 3 weeks during which she received ε ACA (fig 3) In the following periods she was given ε ACA during 9 to 13 days and then the loss of blood was not abnormal The haemoglobin value remained unchanged

All together this patient has received 2958 g ε-ACA in the course of 18 months. She has tolerated the drug well No side reactions whatsoever have been observed

Case 20 born 1947 This was a 17 year-old girl with diabetes mellitus acquired haemolytic anaemia and idiopathic *thrombocytopenia* with epistaxis and gingival bleeding She had since the menarche had profuse menstrual bleeding lasting for about 10 days. She was always anaemic with haemoglobin values ranging between 9—11 g/100 ml and despite increased haemolysis a low serum iron value namely about 40 μg/100 ml The platelet count varied between 4 000—10 000/mm³ The Duke bleeding time varied between 5 and 20 minutes The Ivy bleeding time was > 30 minutes Other coagulation factors were normal

In April 1963 ε ACA therapy was started during the menstrual periods (fig 4) The bleeding decreased considerably and did not last for more than 2 to 4 days Usually 6 to 13 oral doses per period proved sufficient She has now been treated during 12 periods The haemoglobin value has increased and is now regularly about 12 and 13 g/100 ml

She has tolerated the drug well

Case 21 born 1933 A 30-year-old para II probably with idiopathic thrombocytopenia She had always been troubled by profuse menstruation but during the last years the bleeding had increased still more She was anaemic with blood values ranging between 8 and 11 g/100 ml Gynaecological examinations revealed nothing abnormal Repeated curettages had no effect on the profuse bleeding On coagulation analysis the only abnormal finding was that the platelet count was 116 000/mm³ The Duke bleeding time was prolonged and varied between 7 and 12 minutes and the Ivy bleeding time between 18 and 21 minutes During 4 periods she was treated with ε ACA in oral doses of 6 g The first day she received 2—3 doses the second day 2 doses the third 1—2 doses

and the fourth 1 dose. The bleeding was completely controlled. She was, however, troubled by nausea.

Case 22, born 1928. A 33-year-old woman with acute *myeloblastic leukaemia*. She had a low platelet count ranging between 8 000 and 22 000/mm³ and prolonged bleeding time. Other coagulation factors were normal and no fibrinolytic activity could be demonstrated in the blood. She had a profuse and prolonged menstrual flow and gastro-intestinal bleeding.

εACA was given orally in doses of 6 g during one menstrual period. On the first day she bled profusely. εACA was started on the second day, on which it was given four times, and continued for 6 days. The bleeding was normalized and the haemoglobin value did not decrease during this period.

Case 23, born 1915. A 48-year-old woman with *thrombasthenia*: defect platelet adhesiveness. She had since early childhood been [illegible] bled by epistaxis, gingival bleeding and prolonged bleeding following trauma and tooth extractions. The menstrual flow had always been profuse and she had always been anaemic with haemoglobin values about 10 g/100 ml. Four deliveries had been complicated by increased bleeding for which she had received blood transfusions. During the last few years the menstrual flow had increased [illegible] but gynaecological examination revealed no abnormalities. [illegible] had no effect. Coagulation analysis showed a platelet defect with prolonged Duke [illegible] bleeding time, pathological prothrombin [illegible] consumption, decreased platelet adhesiveness and decrease of platelet factor 3. The platelet count was normal as well as other coagulation factors.

She was treated with εACA in oral doses of 5 g during 8 menstrual periods. The bleeding decreased but control of the bleeding required 4 oral doses daily during 4 to 5 days and 2 to 3 doses per day for a further 3 to 4 days. She was troubled by nausea and dizziness. Before treatment was started the haemoglobin value was 8.9 g/100 ml and subsequently rose to 12.8 g/100 ml.

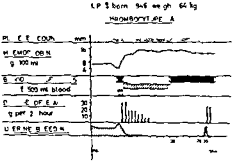


Fig. 5. Administration of εACA to case 24 (I. P.) with profuse menstruation and thrombocytopenia.

Case 24, born 1946. A 17-year-old girl was admitted to hospital because of profuse menstrual flow. The haemoglobin value was 5.2 g/100 ml. Investigation revealed severe *thrombocytopenia* with a platelet count of 2 000/mm³. The bleeding time was 60 minutes. Other coagulation factors were normal. With the exception of the anaemia the findings on blood analysis were normal. Gynaecological examination revealed no abnormalities. She received 5 blood transfusions and steroid therapy was started. The uterine bleeding continued and after 8 days she went into shock (Fig. 5). εACA therapy was then started and given orally, doses of 6 g five times daily during two days. The bleeding decreased considerably. During the following three days she received 3 doses daily, then 2 doses per day on 2 days and one dose per day on 2 days. The bleeding stopped. The steroid therapy was continued but the platelet count increased only to 40 000/mm³. One month after admission renewed profuse menstruation bleeding started. She received 6 g εACA four times daily on [illegible] days. The bleeding was controlled and she required no blood transfusions. Splenectomy was then performed and the platelet count and the menstrual flow became normal.

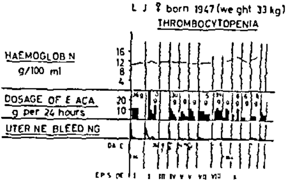


Fig 1 Administration of ε ACA to case 20 (L J) with profuse menstruation and thrombocytopenia

At *period II* the patient received one plasma transfusion and then ε ACA for 3 days The bleeding was controlled On the fifth day ε ACA was withdrawn and she then started to bleed again After ε ACA the bleeding again stopped

At *period I* she also started to bleed when ε ACA was withdrawn after 3 days and a further dose had to be given During the following three periods it proved necessary to continue treatment with ε ACA for 7 to 11 days

In the summer months (*periods IV V and VI*) the bleeding was not profuse During *periods VII and VIII* the patient was treated with repeated transfusions of plasma which controlled the bleeding

During *period VII* treatment with ε ACA every other day was tried but proved insufficient During *periods VI and VII* she bled profusely, and the bleeding did not stop until a daily dose of 30 g of ε ACA had been given

On Dec 22 she had then not bled for 2 days she was allowed to take a walk in the city During her walk she began to bleed profusely and went into shock Five blood transfusions were given She continued to bleed for 3 weeks during which she received ε ACA (fig 3) In the following periods she was given ε ACA during 9 to 13 days and then the loss of blood was not abnormal The haemoglobin value remained unchanged

All together this patient has received 2958 g ε ACA in the course of 18 months She has tolerated the drug well No side reactions whatsoever have been observed

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In April 1963 ε ACA therapy was started during the menstrual periods (fig 4) The bleeding decreased considerably and did not last for more than 2 to 4 days Usually 6 to 13 oral doses per period proved sufficient She has now been treated during 1? periods The haemoglobin value has increased and is now regularly about 12 and 13 g/100 ml

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tion defect but nevertheless responded to ε ACA therapy, also indicate that the profuse menstrual bleedings are due, at least in part to a local release of plasminogen activator

That the local fibrinolysis is an important causal factor of uterine bleeding is evident from the findings in the 6 cases of profuse menstruation due to haemorrhagic diathesis One of these patients was a young girl with a severe bleeding disorder caused by lack of an unknown plasma factor one was a woman with thrombasthenia and 4 had thrombocytopenia The girl with the plasma defect (case 19) lost large amounts of blood and on 2 occasions she went into shock Hysterectomy had not been considered because of the risk of bleeding The menstrual flow in this case was normalized by daily administration of 400—800 ml of plasma or 1—2 blood transfusions But it proved difficult to continue such treatment However ε ACA alone proved sufficient to normalize bleeding, although the patient required rather large doses (75—420 g per period) In case 24 the blood loss was also so heavy that the patient went into shock and here too ε ACA produced a dramatic effect The remaining 4 patients with haemorrhagic diathesis also responded favourably to ε ACA Thus all patients with profuse menstruation and heamorrhagic diathesis responded to ε ACA

The heavy uterine bleeding in these cases of haemorrhagic diathesis could thus probably be explained by the assumption that the primary clotting defect is accentuated by the normally occurring local fibrinolysis in the uterus Elimination of one of these two causes is enough to normalize bleeding ε ACA is therefore useful in the treatment of profuse menstruation in haemorrhagic diathesis and can considerably improve the situation of such patients

No serious side effects were observed Of the 24 patients treated, 15 were troubled by dizziness and/or nausea Only one of them had diarrhoea Owing to these side effects 7 of the patients (cases 2 3 10 11, 12 14 and 17) refused to continue treatment It should be noted that 5 of the 6 patients with haemorrhagic diathesis who received large doses of ε ACA *experienced no* side reactions whatsoever None of the patients treated developed thrombosis The fibrinogen level *did not* rise No toxic effects of the drug on the blood picture were observed

An *important* question is whether treatment with ε ACA can have untoward effects on the foetus during its first 3 *months of intrauterine* life in mothers unware of their pregnancy None of our patients were *pregnant* They *were* also instructed not to start taking ε ACA until the menstrual flow had become profuse and not *try to* prevent *the* flow by taking it before expected menstruation Two *of our* patients (cases 6 and 8) became *pregnant* after treatment with ε ACA and both gave birth to normal children Two similar cases instances *are also* known to have occurred at other hospitals

It may thus be concluded that ε ACA is a safe and valuable drug in the symptomatic treatment of profuse menstruation whether idiopathic or secondary to haemorrhagic disorders

Discussion

In none of the treated patients did gynaecological examination reveal any abnormalities. Most of them had been subjected to curettage but without the desired effect. Except in one case (No. 9) the patients had had profuse menstruation ever since the menarche. The blood losses were not measured, but they had invariably caused anaemia which could not be corrected by iron treatment. The 18 patients described had no other demonstrable bleeding symptoms and were otherwise healthy. The coagulation factors were normal. There was no increased fibrinolytic activity in the blood, and the inhibitors of the fibrinolytic system were also normal. ε-ACA invariably reduced the menstrual flow to normal (table II). The patients were never given ε-ACA until the flow had begun to become profuse. ε-ACA was given in oral doses of 1 g per 10 kg body weight. Bleeding usually began to decrease within one hour. The effect of one dose lasted between 4 and 12 hours. It varied from patient to patient and in some of them from one period to another. When the effect of a dose had worn off and the flow again tended to become profuse, a second dose was given. As a rule, the patients required 2 to 4 doses on the first day, 2 to 4 on the second and 1 or 2 on the third. It was usually not necessary to give ε-ACA on the fourth day.

In previous works (15, 16) we have shown that relatively large doses of ε-ACA are required to produce an effective level of inhibitory activity in the blood and a therapeutic effect. As an adequate dosage we considered 1 g of ε-ACA per 10 kg body weight given every 4—5 hours. In most of these women the effect of one dose lasted longer, i. e. 6 to 12 hours. In some of our patients (cases 3, 4 and 8) with profuse menstruation we tried smaller doses of ε-ACA, i. e. 0.5 g per 10 kg body weight, but the bleeding did not respond satisfactorily and ε-ACA had to be administered at shorter intervals.

One patient (No. 5) has been treated during 19 periods, 5 patients during 5 to 9 periods and the remaining 12 during 1 to 4 periods. The amount of ε-ACA administered during each period has varied from 6 to 90 g. We did not determine the menstrual blood loss as described by Hallberg and Nilsson (12). As already pointed out, all our patients had iron deficiency anaemia before treatment. In our cases treatment for some periods with ε-ACA normalized the haemoglobin level. This suggests that the blood loss had diminished.

Tentative withdrawal of ε-ACA during treatment of cases 5, 10, 13, 14 and 17 regularly resulted in profuse bleeding.

It is obvious that ε-ACA normalize bleeding in cases of profuse menstruation without any known gynaecological disorder. Albrechtsen and Skjodt (3) recently described 15 cases of uterine bleeding treated with ε-ACA. These cases received treatment only during one menstrual period. As mentioned in the introduction Albrechtsen (2) found that human endometrial tissue contains an activator of plasminogen. He believed that this tissue activator was of significance for the occurrence of uterine bleeding. The observations in our patients who had no systemic coagula-

tion defect but nevertheless responded to ε ACA therapy, also indicate that the profuse menstrual bleedings are due at least in part, to a local release of plasminogen activator

That the local fibrinolysis is an important causal factor of uterine bleeding is evident from the findings in the 6 cases of profuse menstruation due to haemorrhagic diathesis One of these patients was a young girl with a severe bleeding di order caused by lack of an unknown plasma factor one was a woman with thrombasthenia and 4 had thrombocytopenia The girl with the plasma defect (case 19) lost large amounts of blood and on 2 occasions she went into shock Hysterectomy had not been considered because of the risk of bleeding The menstrual flow in this case was normalized by daily administration of 400—800 ml of plasma or 1—2 blood transfusions But it proved difficult to continue such treatment However ε ACA alone proved sufficient to normalize bleeding although the patient required rather large doses (75—420 g per period) In case 24 the blood loss was also so heavy that the patient went into shock and here too ε ACA produced a dramatic effect The remaining 4 patients with haemorrhagic diathesis also responded favourably to ε ACA Thus all patients with profuse menstruation and heamorrhagic diathesis responded to ε ACA

The heavy uterine bleeding in these cases of haemorrhagic diathesis could thus probably be explained by the assumption that the primary clotting defect is accentuated by the normally occurring local fibrinolysis in the uterus Elimination of one of these two causes is enough to normalize bleeding ε ACA is therefore useful in the treatment of profuse menstruation in haemorrhagic diathesis and can considerably improve the situation of such patients

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An important question is whether treatment with ε ACA can have untoward effects on the foetus during its first 3 months of intrauterine life in mothers unware of their pregnancy None of our patients were pregnant They were also instructed not to start taking ε ACA until the menstrual flow had become profuse and not try to prevent the flow by taking it before expected menstruation Two of our patients (cases 6 and 8) became pregnant after treatment with ε ACA and both gave birth to normal children Two similar cases instances are also known to have occurred at other hospitals

It may thus be concluded that ε ACA is a safe and valuable drug in the symptomatic treatment of profuse menstruation whether idiopathic or secondary to haemorrhagic disorders

Summary

We have administered ε-ACA to 24 females with profuse menstrual flow but without any demonstrable gynaecological disorder. The blood losses had invariably caused iron deficiency anaemia. Eighteen of these patients were otherwise healthy women and 6 suffered from a haemorrhagic diathesis. Of the 18 women with normal coagulation mechanism one was treated during 19 periods, 5 during 5 to 9 periods and the remaining 12 during 1 to 4 periods. ε-ACA invariably reduced the menstrual flow to normal. It was given in oral doses of 1 g per 10 kg body weight but not until the flow had become profuse. The amount of ε-ACA given during each period varied from 6 to 90 g. Treatment for some periods normalized the haemoglobin level. Two patients became pregnant after treatment and gave birth to normal children.

In the 6 patients with haemorrhagic diathesis (v. Willebrand like disease, thrombasthenia, thrombocytopenia) and profuse menstruation ε-ACA, alone, proved sufficient to normalize bleeding, although some of the patients required rather large doses (75—420 g per period). In two patients who went into shock owing to the large blood loss, the response to ε-ACA was dramatic. One of these patients was treated during 19 periods and received 2958 g ε-ACA without any side reactions. It is suggested that the bleeding caused by the primary clotting defect is enhanced by the normally occurring local fibrinolysis in the uterus.

No serious side effects were observed.

It is concluded that ε-ACA is a valuable drug in the symptomatic treatment of profuse menstruation, whether idiopathic or secondary to haemorrhagic disorders.

Acknowledgement

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Cutis Verticis Gyrata and Mental Deficiency in Sweden

II Genetic Aspects

By

Hans Olof Åkesson

Cutis verticis gyrata occurs in several different and probably unrelated, syndromes Familial occurrence is seldom reported except when it is a component of the syndrome pachydermoperiostosis Pachydermoperiostosis is genetically conditioned though it is still not clear how it is transmitted one often finds it among the sibs of one s patients occasionally among their parents as well

Spinz (4) and Vogeli (6) both observed cutis verticis gyrata in an otherwise unaffected father and son Truffi (5) observed a combination of cutis verticis gyrata and mental retardation in two members of one family Judging from the literature however hereditary occurrence of the scalp disorder is not common On the other hand the literature on the subject consists almost entirely of descriptions of one or a few cases and no one has published a systematic genetic analysis of the condition

I have now made an analysis of this kind based on all the cases of cutis verticis gyrata occurring among the mentally retarded subjects institutionalized in Sweden in 1961 and 1962 They amounted to 47, and contained 46 men and 1 woman I have already reported the observations of epidemiologic and clinical interest in this series (2) The present paper deals with the data of genetic interest

The 47 subjects are described in my previous report (2)

Submitted for publication September 29 1964

Parental age and birth rank

As known non genetic factors may influence when affected subjects are born in relation to the parents age Non genetic factors may also affect the distribution of the subjects by birth rank In other words if a disorder stems wholly from genetic factors, affected persons should not differ from people as a whole in parental age at birth or birth rank

To see whether the present series deviated from the general in parental age

the average when the patients were born and 28 3 years old when the controls were born Likewise this is not a significant difference (t = 1 6 88 d f 0 10 < P < 0 20)

Because of the P value (P = 0 05) for the difference in paternal age between patients and controls, I compared the patients with a control series elected in another way This series consisted of men and women from the general Swedish population who produced children the same year the patients were born

Table I shows the paternal age at birth for the patients and this second control series (expected values) The difference between the two groups was not significant (χ^2 = 3 7 1 d f 0 05 < P < 0 10) Table II shows the same for the maternal age Nor does this table show a significant difference (χ^2 = 0 88 1 d f, 0 30 < P < 0 50)

As seen from table III the 47 probands were randomly distributed by birth rank The difference between the total observed and expected figures is not significant (χ^2 = 3 3 3 d f, 0 30 < P < 0 50)

Thus the present series did not show a statistical connection between cutis verticis gyrata and parental age or birth rank

Morbidity among parents and sibs

I collected data on the morbidity among parents and sibs by studying all the available medical records of interest and by interviewing relatives in detail

Four out of the 88 parents examined or 4 5 ± 2 2 per cent (4 fathers and 2 mothers were unknown), showed mild mental deficiency This rate does not differ significantly from that for the general population, which I have estimated to be between 1 and 2 per cent (1) No parent was severely retarded

One parent had schizophrenia, 1 arteriosclerotic dementia and 2 epilepsy None had cutis verticis gyrata, cerebral palsy or eye defects apart from 1 case of strabismus

Thus the morbidity among the parents did not differ significantly from that in the general population

Before analyzing the morbidity among the sibs, I excluded all the sibs under 5 or 2 boys and 2 girls This left 138 sibs None of these had cutis verticis gyrata But 5 male and 3 female sibs or 5 8 ± 2 0 per cent, were severely retarded, having an I Q of 25 at the most According to an estimate I made previously (1), 0 5 per cent at the most of the general Swedish population have I Q 's of 25 or less, probably less than 0 3 per cent Thus severe mental deficiency was significantly over represented among the sibs of the patients According to the Poisson distribution with a prevalence of 0 5 per cent in the general population the probability of 8 out of 138 being affected randomly is less than 0 005

Sex ratio

A combination of cutis verticis gyrata and mental deficiency is extremely rare among women The present series consisted of 46 men and 1 woman All 47 patients belonged to different sibships and the total number of persons in these sibships including the patients amount

TABLE I Fathers of 43 mentally deficient persons with cutis verticis gyrata by age at birth of the affected child

Paternal age (years)	Observed no of fathers	Expected no[1] of fathers
25	3	3.0
25–30	10	10.1
30–35	7	11.3
35–40	8	8.9
40–45	12	5.6
>45	3	4.1
Total no	43	43.0

[1] Data from official Swedish statistics (see article)

TABLE II Mothers of 45 mentally deficient persons with cutis verticis gyrata by age at birth of the affected child

Maternal age (years)	Observed no of mothers	Expected no[1] of mothers
15–20	3	0.7
20–25	6	8.1
25–30	11	12.7
30–35	10	11.3
35–40	11	8.1
40	4	4.1
Total no	45	45.0

[1] Data from official Swedish statistics (see article)

TABLE III Birth rank of 47 mentally deficient persons with cutis verticis gyrata

Size of sibship	Number of affected individuals by rank												Expected no each rank
	1	2	3	4	5	6	7	8	9	10	11	12	
1	(11)												(11)
2	3	2											3
3	1	1	3										8
4	1	1	2	1									5
5	—		–	3	3								6
6	1		1	1	1	—							4
7		1		1		1	—						5
8	–	—		1				1					2
9	1						—						1
12	1				1	—	—				—		2
Total obs	11	5	6	7	5	1		1					36
Total exp	9.24	9.24	6.74	4.07	2.82	1.62	0.96	0.53	0.28	0.17	0.14	0.1	36.01

I compared the parental age at birth for the present subjects with that for the persons of the same sex born directly after them in the same parish. The paternal age was unknown for 4 of the patients and the maternal age for 2.

The fathers were 34.9 years old on the average when the patients were born and 31.7 years old when the controls were born. The difference is not significant ($t = 2.0$, 84 d f, $P \simeq 0.05$).

The mothers were 30.4 years old on

TABLE IV Comparison between numbers of affected (aborted sibs included) and healthy sibs observed with numbers which could be expected with a recessive mode of inheritance according to the Apert Bernstein method

Sibship size	No of families	Affected sibs		Unaffected sibs	
		Obs no	Exp no	Obs no	Exp no
2–4	9	14	12 04	15	16 96
5–6	5	12	8 57	15	18 43
7–10	5	11	11 55	31	30 45
Total	19	37	32 16	61	65 84

recessive mode of transmission Against this hypothesis is the fact that the father of the female proband was not affected True, this may have been because the sex cells of the father had mutated or the penetrance had decreased On the other hand a sex linked recessive gene would not explain the excess number of males among the healthy sibs

A recessive autosomal mode of inheritance is compatible with the relatively large number of first cousin marriages and with no parents but many sibs being affected As a rule little is gained by speculating on how a disorder is inherited but the fact that so few women were affected should permit one to theorize along the following lines

The most probable explanation for *the sex disproportion among the patients* is that in these families the female fetuses were aborted more often than were the male fetuses It may be that the disease incurs an increased risk of female fetuses being aborted Other explanations such as the presence of sex determining factors seem to me to be less plausible

I estimated the frequency of abortion in 19 families on the basis of testimony from the 19 mothers According to them 13 out of their 101 pregnancies, or 13 ± 3 3 per cent ended in abortion Whether this rate differs from the normal is impossible to say as reliable data are lacking on this point Five of the 13 abortions occurred late in pregnancy It was possible to get figures for the birth weight for only a few of the patients as most were delivered at home It is noteworthy however that 9 of the 27 mothers questioned on this point, or 33 *per cent* said that the patient was born too early and was very small It is probable therefore that the syndrome *incurs* a large risk of abortion especially for female fetuses

Assuming that the aborted fetuses were affected analysis of the manner of transmission in the 19 families by the Apert Bernstein method (table IV) gives results tallying well with recessive inheritance ($\chi^2 = 2 6$ 3 d f, $0 30 < P < 0 50$ for the difference between *observed* and expected *figures*) The frequency of affected sibs calculated

ed to 189, of these 133 were males and 56 females

There was also a significant disproportion between the sexes among the sibs, there being 87 males and 55 females ($\chi^2 = 7.2$, 1 d f, $P < 0.01$) Even when the 8 severely retarded sibs were left out, the disproportion remained statistically significant ($\chi^2 = 6.7$ 1 d f, $P < 0.01$)

Thus the present material shows a statistically significant disproportion between the sexes both among the patients and among their healthy sibs

Discussion

As reported in my previous article (2) I estimated that 8 per cent of the probands were the offspring of first cousins, as opposed to 1 to 2 per cent in the population in general (1) None of the patients appeared to have any ancestor in common

Analysis of the chromosomes in the peripheral leucocytes of 1 patient showed a normal male karyotype Berg and Windrath Scott (3) also observed a normal chromosome pattern in a mentally deficient patient with cutis verticis gyrata Study of the sex chromatin in buccal smears from 14 of the present male patients showed no sign of sex chromatin bodies (2) The female patient showed the sex-chromatin pattern normal for females

To sum up the data of genetic interest in the present series 1) the sibs of the patients showed an excess rate of severe mental deficiency 2) the parents of the patients did not differ from the general population in intelligence, 3) the patients seemed to be more often the offspring of first-cousin marriages than people in general, 4) the patients did not deviate from the average in parental age at birth or birth rank 5) studies of the chromosomes and sex chromatin gave normal results, 6) both the patients and their healthy sibs contained a significantly lower number of females

The excess rate of severe mental deficiency among the sibs of the patients points to the operation of genetic factors Five sibships contained more than one retarded subject, and the deficiency was of the same degree in the affected sibs as in the patients I took only the parents and sibs into account in the genetic analysis, and did not make a systematic study of the other relatives According to the records available however, 15 relatives now dead were also severely retarded (altogether 12 families contained 2 or more severely retarded persons) It seems likely, therefore, that the syndrome may stem from hereditary factors

As mentioned, none of the 8 severely retarded sibs had cutis verticis gyrata But the scalp disorder sets in late in life often only a few years before the patient dies (2) Moreover the 47 cases of the scalp disorder were taken from a mentally deficient population The clinical analysis revealed that these 47 often had other physical disorders as well notably epilepsy, cerebral palsy and eye defects though not consistently Thus variation in the expression of the syndrome may also explain why none of the 8 affected sibs had the scalp disorder

The disproportion between the sexes among the patients suggests a sex linked

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A Family with Mild Diabetes Mellitus, Hyperlipemia and Atherosclerosis

By

HELGE SIGSTAD

In 1955 Adlersberg and Wang described the syndrome of idiopathic hyperlipemia mild diabetes mellitus and severe vascular damage (1) Three of the five persons observed had a history of diabetes in their family Idiopathic hyperlipemia associated with mild diabetes must be *differentiated from the* secondary symptomatic form of hyperlipemia occurring in severe uncontrolled diabetes (1 6) A combination of both forms of hyperlipemia occurs in some cases of diabetes (1) The present investigation presents data from a study of a family with 9 cases of mild diabetes mellitus two cases of hyperlipemia and several cases of atherosclerosis

Methods

All examinations of the lipid content in serum were done on specimens collected after an overnight fast Total serum lipids were determined by a gravimetric method after extraction with ether alcohol values above 1 200 mg 100 ml were regarded as definitely abnormal Total cholesterol was determined by the method of Klungsøyr et al (15) Total phospholipids were determined by phosphorus analyses and the values are expressed as phospholipid by using a commonly accepted mean value for phosphorus as 4 % of the total weight of the phospholipids The fraction called neutral fat and free fatty acids etc was calculated by subtracting the figure for cholesterol plus phospholipids from that for the total lipids The glucose concentration in blood was determined according to Hagedorn and Jensen (11) on capillary blood Intravenous glucose tolerance tests were performed according to Lundbaek (17) The latter two analyses were carried out in the clinical laboratories of the medical departments On Aug 20 1963 the serum lipids were determined at the Institute for Clinical Biochemistry University Hospital Rikshospitalet Oslo The total lipids were determined gravimetrically after extraction according to Schoenheimer and Sperry (19) Total cholesterol was determined by the method of Carr and Drekter (5) Free cholesterol was determined by the method of Carr and Drekter after extraction according to Schoenheimer and Sperry and precipitation of free cholesterol with digitonin Lipid phosphorus was determined by the method of Fiske and

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with Weinberg's proband method (0.23 ± 0.05) also tallies well with what could be expected with recessive inheritance (0.25). Both affected children and abortions were counted as affected in these calculations. But it does not follow from autosomal recessive inheritance that female fetuses are more apt to be aborted.

In conclusion, I wish to emphasize that none of what I have now said allows any conclusion about the way the syndrome is inherited. It is impossible to judge the part played by external factors in a combination of cutis verticis gyrata and mental deficiency. Furthermore, it may be that the combination occurs in syndromes of different origins.

Summary

A series of 17 patients with a combination of cutis verticis gyrata and mental deficiency, 16 men and 1 woman, yielded the following data of genetic interest. (The epidemiologic and clinical data have been previously reported.)

The sex chromatin and chromosome patterns were normal. The same was true of the parental age and birth rank.

About 8 per cent of the patients were the offspring of first cousin marriages. The parents did not differ from the general population in morbidity. The sibs contained an excess number of severely retarded subjects.

There were significantly few females both among the patients and among their healthy sibs. The most plausible explanation for this seemed to be that the female fetuses in these families were more often aborted than were the male fetuses, the disease probably incurring an increased risk of abortion for female fetuses.

The syndrome cutis verticis gyrata was concluded to be genetically conditioned. Different modes of transmission were discussed, but no definite conclusion could be drawn. It was emphasized that it is impossible to judge the part played by external factors, and pointed out that the syndrome might be of several different origins.

Acknowledgement

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TABLE I Fasting serum lipids fasting blood sugar and K value

Case	Date	Total lipids (mg/100 ml)	Total cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Neutral fat and free fatty acids etc (mg/100 ml)	Fasting blood sugar (mg/100 ml)	K value (intravenous glucose tolerance)
6	5 10 62	3 235	3[illegible]0	620	2 225	205	
	30 10 62	1 400	337	168	895	117	
7	12 1 63	1 215	211	183	821	259	
8	22 3 60		330			115	
9	13 6 55		327			86	
11	9 6 59	3 305	863	840	1 602	241	
	13 4 64	1 000	384	128	488	105	0 97
12	2 10 62	1 400	328	195	8[illegible]7		
	6 4 64	1 080	311	178	591	112	0 83
13	2 10 62	1 060	264	185	611		
	27 4 64	1 0[illegible]0	302	143	600		1 78
14	2 10 62	1 230	303	230	697		
	6 4 64	1 000	306	200	4[illegible]4		1 54
15	6 3 62	720	229	113	378		
	17 1 64	800	221	165	414		1 [illegible]4
K S	7 3 62	1 0[illegible]0	217	156	677		
N S	7 3 62	1 060	271	146	643		
	17 1 64	740	230	110	400		2 10

symptoms of diabetes In 1962 on admission to hospital hyperlipemia and a mild diabetes mellitus were revealed

Case 7 had xanthelasmata palpebrarum and a mild diabetes mellitus for the last 10 years of her life She was hospitalized in Jan 1963 because of myocardial infarction and died 4 days later At autopsy generalized arteriosclerosis was found The basal vessels of the brain showed considerable atheromatous changes and a high degree of calcification In the aorta there was considerable atheromatosis with calcification and extensive ulceration The coronary arteries also showed considerable atheromatous changes and the ramus circumflexus of the left coronary artery was completely occluded near its origin Microscopic examination showed fibrosis and hemorrhage in the anterior part of the wall of the left ventricle (The Gade Institute Department of Pathology Head E Waaler M D)

Case 8 is a man having suffered from a mild diabetes mellitus for some years He has arcus senilis and peripheral atherosclerosis

Case 9 had for six years diabetes mellitus treated with insulin During her last years she suffered from an *increasing cerebral* atherosclerosis

Case 10 was in good health until she died in child bed when 27 years old

Case 11 is the eldest daughter of case 6 with hyperlipemia She also had *hyperlipemia* which was the first *symptom* of the disease On examining her sedimentation rate the

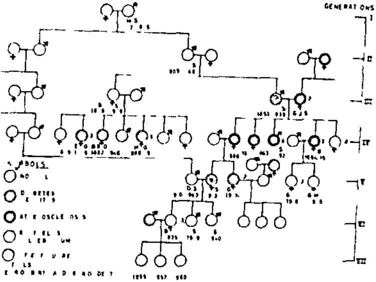


Fig. 1 a. Family tree.

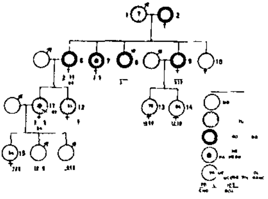


Fig. 1 b. The family members investigated and some of the results.

Subbarow (9). Triglycerides were determined by the method of Van Handel and Zilversmit (21) and non esterified fatty acids by the method of Dole (7) as modified by Trout et al. (20).

The family tree is demonstrated in figs. 1 a and 1 b. An apparently negative family history in the two first generations does not necessarily exclude the occurrence of diabetes. The members of these two generations have not been adequately examined because at that time diagnostic urine- and blood sugar analyses were not done. It was not until the end of the nineteenth century that routine tests for sugar in urine became a common practice (4). The first practical method for blood sugar determination was described by Bang in 1913 (3). This means that definite proof of the existence of diabetes in the family can hardly be obtained for more than the last five generations.

Case reports

Case 1 (figs. 1 a and 1 b) suffered from polydipsia and drank 4 liters sour milk a day the last years of his life, but never consulted a doctor.

Case 2 died of heart attack. In her side of the family no case of diabetes is known but several ancestors developed early cerebral atherosclerosis, among those her mother, who had recurrent apoplectic insults.

Cases 3, 4 and *5* were unmarried sisters who had all a mild diabetes mellitus. Two of them got their diabetes after the age of 50. They all died several years ago.

Case 6 suffered for several years from an increasing cerebral atherosclerosis but had no

decreased to nearly normal values and her elevated fasting blood sugar was normalized Her body weight was reduced to normal, 55 kg and remained constant when the patient received a diet consisting of 1,700 calories a day Dietary fat furnished 27 per cent of total calories Polyunsaturated fatty acids made up 8 6 per cent of total fat Fifty five per cent of calories were derived from carbohydrate and 18 per cent from protein During this treatment the total serum lipids gradually increased due to increase of neutral fat and free fatty acids etc A marked incresae was observed in December 1961 probably the result of a higher fat intake during Christmas

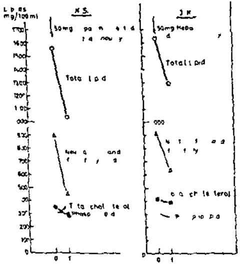


Fig 3 The effect of intravenous heparin injection on the serum lipids in case 11 (K S) and case 6 (J K)

After intravenous injection of 50 mg of heparin the total lipids one hour after injection were reduced (fig 3) The reduction was 3 2 g/l reckoned to be 13 g in all This decrease was caused by a reduction of neutral fat and free fatty acids etc while cholesterol and phospholipids remained almost unaltered In fig 3 a similar decrease in the lipid values is demonstrated in case 6 who is the mother of case 11

The effect of a single intravenous injection of heparin however is transient Fig 4 shows the lipid curves after a single

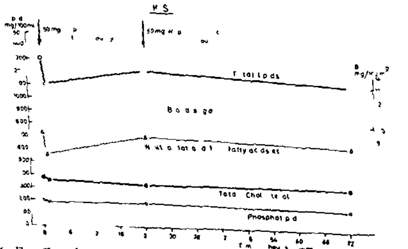


Fig 4 The effect of intravenous injection of heparin on the serum lipids and the blood sugar in case 11 K S determined during three days

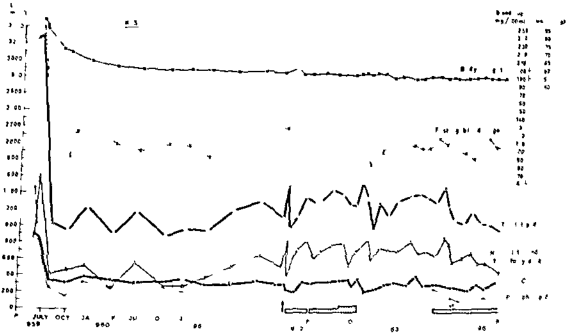


Fig. 2. Body weight, fasting blood sugar and fasting serum lipids in case 11 from May 1959 to April 1964. The treatment used is shown on the diagram.

doctor noticed that her plasma looked quite milky. On admission to hospital in 1959 a milky appearance of the serum from a fasting morning specimen was noted. A mild diabetes mellitus was detected but she did not complain of any subjective symptoms indicative of diabetes. She had xanthelasmata palpebrarum but she showed no clinical signs of atherosclerosis. She is related to her husband (fig. 1 a).

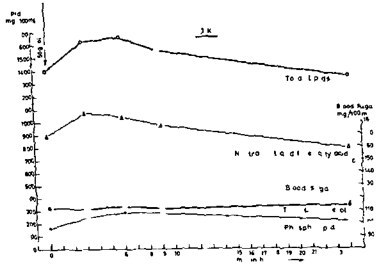
Case 12 has no subjective symptoms of diabetes or other diseases. An intravenous glucose tolerance test, however, showed the value 0.83 signifying a diabetic state. An elevation of neutral fat and free fatty acids etc. was observed. This lipid fraction, however, decreased during treatment with a low carbohydrate diet (table I). Her non-related husband may also be a carrier of diabetes mellitus because his mother suffers from the disease.

Case 14 had total serum lipids that bordered the normal upper limit. On control her serum lipids showed normal values (table I). Meanwhile she is on a low caloric diet. An intravenous glucose tolerance test gave the value 1.54 suggesting a non-diabetic state. She is regarded as an observation case to be followed by repeated tests.

Case 15 is a non-diabetic whose non-related husband has a juvenile diabetes which requires the use of insulin.

Results

Case 11 is the most interesting one in the family. On admission to hospital in June 1959 she was obese: weight 85.2 kg, height 156 cm. There was a marked elevation of total lipids which was due to elevation of cholesterol, phospholipids and the fraction consisting of neutral fat and free fatty acids etc. Fig. 2 presents data from a five year study of the fasting serum lipids and the fasting blood sugar in this patient. She received a low caloric low-fat diet. During this treatment her fasting serum lipids rapidly



F g 7 Fat tol rance test in case 6 (J K At zero t me 50 g ol ve-o l was fed Thereafter fat free formula was fed throughout the day

Total lipids 1 470 mg/100 ml Total cholesterol 316 mg/100 ml Free cholesterol 101 mg/100 ml Phospholipids 343 mg/100 ml Triglycerides 400 mg/100 ml NEFA non esterified fatty acids) 1 61 mEq/l

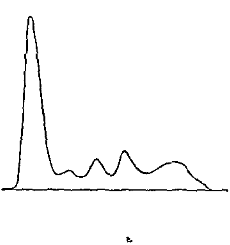
Almost corresponding results were btained simultaneously at the two laboratories (fig 2)

A fat tolerance test performed in case (fig 7) shows an essentially normal curve A significant rise in the blood sugar was noted during the test before the patient had received carbohydrate

Electrophoretic separation of the serum l poproteins (fig 8) shows an increase of β lipoprotein The triglycerides are mainly attached to the lipoprotein fraction which contains the very low density l poprotein

The coagulation factors were found rmal at the Institute for Thrombosis Research University Hospital Riks h spitalet Oslo

Prothrombin Proconvertin 74 % Quick time 14 3 sec Cephalin time 59 2 sec Proaccelerin 125 % Stuart Prower factor 96 % Fibrinogen 270 mg/100 ml Antihemophilic A factor 74 %



F g 8 The electrophoretic pattern in case 11 (K S

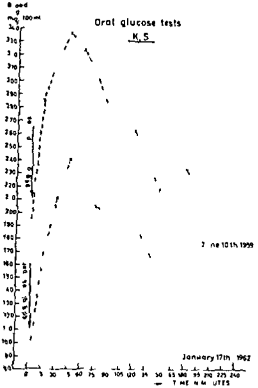


Fig. 5. Oral glucose test in case II (K. S.). The first test was performed in June 1959 when the patient was obese and hyperlipemic. The second test was performed in Jan. 1962 when the weight and the serum lipids were normalized.

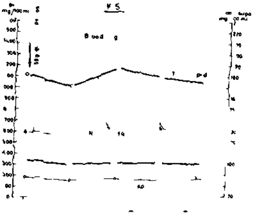


Fig. 6. The effect of an oral glucose tolerance test on the serum lipids in case II (K. S.).

intravenous injection of 50 mg of heparin. The lipid values again rise to nearly pre-injection level on the next morning. After the injection of heparin a significant fall of the blood sugar is noted coincident with the fall of the serum lipids. An insulin tolerance test showed normal insulin sensitivity.

For two months the patient received 15 g of soybean oil per day which replaced 15 g of margarine. Polyunsaturated fatty acids then made up 24.8 per cent of the total fat in this diet. This addition of polyunsaturated fatty acids to the diet did not alter the lipid values significantly.

From April 1962 our patient received subcutaneous injection of heparin in aqueous solution, at first 250 mg twice a week and later the same dose was given every second day. The patient was treated with heparin for a period of 6 months. No significant changes in the serum lipid pattern were observed. During the treatment, however, her xanthelasmata disappeared. No untoward reactions to the heparin were observed.

From July 1963 our patient has been treated with 3-pyridineacetic acid 1,000 mg twice a day. During this treatment there seems to be a decrease of the total lipids caused by a reduction of the *neutral fat and free fatty acids etc.* (fig. 2).

The carbohydrate metabolism was investigated by oral glucose tolerance tests. One gram of glucose per kilo body weight was given at zero time (fig. 5). Intravenous glucose tolerance test showed the value 0.97 which is not significant for a diabetic state (17). Fig. 6 demonstrates the effect of an oral glucose tolerance test on the serum lipid values. These show only small variations.

On Aug. 20, 1963 the serum lipids were determined at the Institute for Clinical Biochemistry, University Hospital Rikshospitalet, Oslo.

He found it reasonable to assume that an accelerated triglyceride influx saturates the reserve capacity of the triglyceride-removal mechanism which in itself is normal

The marked elevation of the free fatty acids in case 11 indicates a hyperlipemia owing to mobilization of depot fat The triglycerides are also considerably elevated while cholesterol and phospholipids show almost normal values This suggests that the hyperlipemia may be due to an elevation of the very low density lipoproteins which is indicative of hyperlipemia of the mobilization type

There has been a growing awareness of the fact that diabetes mellitus is a general complex fundamental disease process with many facets (8) The aberration of carbohydrate metabolism is but one facet Many complications of diabetes may occur in the absence of manifest diabetes (8) Case 6 suffered for several years from an increasing atherosclerosis cerebri before a mild diabetes mellitus was revealed As mentioned by Wahlberg (23) it is conceivable that subclinical diabetes mellitus predisposes to vascular disease in the same way as clinical diabetes mellitus Wahlberg's preliminary investigation rises the question whether the primary disease of the atherosclerotic patients with pathological glucose tolerance is subclinical diabetes mellitus In trying to get an early diagnosis of diabetes mellitus the fasting blood sugar value is entirely inconclusive and may be misleading (table I) A glucose tolerance test should preferably be performed

In the family described above the metabolism of both carbohydrate and fat is abnormal We have found a connection between the hyperlipemia and the hyperglycemia in our two hyperlipemic diabetics (fig 2, table I) Hirsch (13) has made the same observation

A number of observations suggests an association between coronary artery disease, elevation of serum triglycerides and abnormal glucose tolerance (2) An increased triglyceride concentration, with either normal or elevated cholesterol and slight to marked turbidity of serum, is the commonest lipid abnormality in coronary artery disease (2) In case 7 the examination of the lipid content was done on serum collected two days after a myocardial infarction However the lipids of patients with recent myocardial infarction are not likely to be meaningful until at least 10 days after the attack because of the depressing effect of the acute illness on serum lipids (2)

The lipid pattern in case 11 (fig 2, table I) may be an indication of potential atherosclerotic disease According to current views on the role of lipids in the evolution of atherosclerosis the maintenance of the blood lipids in diabetes at or near the high normal range is significant in delaying the evolution of degenerative vascular lesions (13) Usually 10–15 years of diabetes mellitus are required for the development of these degenerative vascular lesions (13) The best way to delay this development in our patients is a control of blood sugar and serum lipids

Rosenman and Friedman (18) studied the effect of administration of heparin in a patient with idiopathic hyperlipemia During 6 months subcutaneous in

Discussion

In the 4th generation of the family studied, seven members had diabetes mellitus and five were non diabetics The disease seems to have a great penetrance As the number of affected and unaffected individuals is nearly equal the inheritance may well be dominant

Only one of the seven diabetics in the 4th generation (no 9) was under treatment with insulin The other six diabetics had a mild form which was easily controlled by diet alone Cammidge (4) points out that the dominant variety of diabetes is almost invariably mild while the recessive form, on the other hand, is generally grave from the onset and almost invariably requires the use of insulin

Six diabetics in the 4th generation had the late-onset form of the disease while in one case (no 3) the disease occurred early in life Harris (12) suggests that many of the late onset cases may be heterozygous for a gene which in homozygous form may give rise to the severe early-onset type of cases

No sign of metabolic disease is found in the individuals in the 6th and the 7th generations Normal glucose-tolerance curves however do not preclude the future development of diabetes (14) The majority of cases of diabetes in the 4th generation were diagnosed after the individuals had passed the age of 50 years It is a matter of opinion whether one should regard an abnormality of carbohydrate or lipid metabolism as the primary defect The first sign of disease discovered in case 11 was the milky plasma At that time she was 46 years old and had no subjective symptoms of diabetes Her fasting blood sugar, however, was elevated At an age of 46 case 12 had no subjective symptoms of diabetes and her fasting blood sugar was 112 mg/100 ml An intravenous glucose tolerance test showed the value 0 83, establishing a diabetic state Eighteen months earlier her total serum lipids were slightly elevated due to an increase in neutral fat and free fatty acids etc (table I)

The two hyperlipemic patients both have a mild mellitus In case 11 the serum lipids initially decreased to nearly normal values during treatment, but later neutral fat and free fatty acids etc increased and were elevated on many occasions in spite of normal values of the fasting blood sugar Examination of the fasting serum lipids may perhaps provide an opportunity for the early recognition of the diabetic syndrome Adlersberg (1) states that while secondary hyperlipemia is completely reversible by adequate treatment of the underlying diabetic disturbance, idiopathic hyperlipemia may be corrected, but rarely reverts to normal After the fasting blood sugar was restored to normal the curve was still diabetic (fig 5) The glucose values in the second curve are approximately 100 mg 100 ml lower than the corresponding values in the first curve Waddell et al (22) carried out glucose tolerance tests in five patients with idiopathic hyperlipemia Each of these curves was abnormal because of a somewhat exaggerated elevation of the blood glucose concentration and because of delayed return to the fasting level Crofford has studied the mechanism of idiopathic hyperlipemia with hyperglycemia (6)

Addendum

Case 11 was treated for one year with 3 pyridine acetic acid 1 000 mg twice a day After a transient reduction of the serum lipids a gradual increase however was observed and the drug was discontinued For 5 months the patient has been treated with the oral hypoglycemic agent chlorpropamide 125 mg daily No change in the serum lipid pattern is observed

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jections of heparin were given twice a week. Repeated analyses showed a progressive reduction of plasma lipid levels which approached normal values. Lever et al. (16) observed that cutaneous xanthomata disappeared or decreased in size in three patients who received heparin for a prolonged period of time. During treatment with subcutaneous injection of heparin the serum lipids did not alter significantly in case 11, but her xanthelasmata palpebrarum disappeared.

The action of 3-pyridineacetic acid on lipid metabolism has lately been studied (10). The drug is considered to be one of the most active available for the correction of the abnormalities of the humoral pattern which very often form the basis of atherosclerosis (10). A probable reduction of neutral fat and free fatty acids was observed during this treatment in case 11.

Case 12 has three children and so has case 15. The children should be followed with special interest in the future. As both the father and the mother may be gene carriers, the expected incidence of diabetes appearing among these six children may be rather high.

Summary

A family with 9 cases of mild diabetes mellitus, two cases of hyperlipemia and several cases of atherosclerosis is described. The inheritance may well be dominant. No symptoms or signs were found in individuals below the age of 45 years. Signs of a mild diabetes mellitus were revealed at an age of 46 years in two sisters. One of them had hyperlipemia, the other showed elevation of neutral fat and free fatty acids. Examination of the fasting serum lipids may perhaps provide an opportunity for the early recognition of the diabetic syndrome. Six individuals in one generation got a mild diabetes mellitus after passing the age of 50 years. A marked atherosclerosis was found in some individuals above the age of 60 years. One of them suffered from an increasing atherosclerosis cerebri for several years before her mild diabetes mellitus was revealed.

One of the hyperlipemic diabetics, who was obese, case 11, was treated successfully with a low caloric, low fat diet. During this treatment her body weight was reduced to normal and her fasting blood sugar and fasting serum lipids were normalized. The patient was then on a diet consisting of 1,700 calories which maintained constant body weight. The total lipids gradually increased by virtue of a marked elevation of the free fatty acids and triglycerides. No significant change in the lipid values was observed when 15 g of soybean oil were substituted for 15 g of margarine a day.

Intravenous injection of heparin produced a transient reduction of neutral fat and free fatty acids etc. During treatment with subcutaneous injection of heparin the serum lipids did not alter significantly. A probable reduction of neutral fat and free fatty acids was observed during treatment with 3-pyridineacetic acid.

The hyperlipemia found in this form of mild diabetes mellitus is probably of the mobilization type.

Addendum

Case 11 was treated for one year with 3 pyridine acetic acid 1 000 mg twice a day After a transient reduction of the serum lipids a gradual increase however was observed and the drug was discontinued For 5 months the patient has been treated with the oral hypoglycemic agent chlorpropamide 125 mg daily No change in the serum lipid pattern is observed

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Hemodynamics of Novadral® in Normal Man

By

M. H. Frick, Timo Somer and Pentti I. Halonen

The proper combatting of shock in its various forms is a frequently occurring challenge in medicine. To overcome the side effects and weak points of action of the natural pressor amines, notably norepinephrine, synthetic compounds have been developed (2). Two of these, mephentermine and metaraminol, have been shown to elicit in man, in addition to peripheral vasoconstriction, an inotropic effect on the heart (8, 9).

Numerous clinical trials have demonstrated the effectiveness of Novadral (m-oxyphenyl (1) ethanol (1) amine (2) hydrochloride; Diwag AG, Berlin-Waidmannslust), a synthetic pressor amine, in various forms of shock and hypotensive states (e.g. 11–13). Slowing of the heart rate and in parallel the rise in blood pressure were the principal findings in these studies. Knowledge of the hemodynamic basis of these changes in man is based, however, on only two studies on pressure-flow relationships. Kaczmarek and Puppe (6), utilizing indirect means to determine the cardiac output, observed a sharp elevation of the peripheral resistance and a reduction of the cardiac output. Their assertion of an increased stroke volume was supported by a figure showing a decline instead. Fasbender (3), using the same technic, was unable to demonstrate an inotropic effect on the heart.

The paucity of hemodynamic data despite the seemingly wide use of Novadral in some countries prompted the present study.

Material and methods

Ten healthy paid volunteers aged 18—20 were studied. The blood pressure was measured via an indwelling Cournand needle from the brachial artery with the aid of Statham pressure transducers (P 23 AA) and photoelectric registration. The mean pressure was obtained by electrical integration. The pressure reference level was the middle of the chest at the level of the second costal cartilage. The subjects were supine throughout the procedure.

Cardiac output (CO) and central blood volume (CBV) were determined by dye

Submitted for publication October 8, 1964.

TABLE I Mean values (± S D) of the various parameters before during and after novadral infusions

	Brachial art pressure (mm Hg) S	D	M	Heart rate (beats/min)	Stroke index (ml/m²)
Before infusion	131 ±14.6	74 ±4.3	87 ±6.9	73 ±10	45.3 ±9.5
During slow infusion	144 ±4.3 <0.05	84 ±6.6 <0.02	102 ±9.2 0.05	61 ±6.5 <0.05	41.6 ±6.8 <0.20
During rapid infusion	159 ±16.8 <0.05	93 ±9.9 <0.02	116 ±7.9 0.02	52 ±3.5 0.05	45.3 ±11.[illegible] <0.05
5 min after infusion	144 ±11.3 0.05	82 ±6.3 <0.05	103 ±7.9 <0.05	63 ±8.3 <0.05	48.0 ±9.[illegible] <0.20
10 min after infusion	142 ±12.6 < 0.05	79 ±6.8 0.05	100 ±6.7 0.05	71 ±5.6 <0.80	50.9 ±13.6 < 0.10
20 min after infusion	139 ±10.0 0.10	77 ±3.5 0.05	97 ±7.7 0.05	72 ±7.6 0.80	52.0 ±12.7 0.10
40 min after infusion	141 ±12.6 < 0.20	77 ±6.2 0.10	93 ±7.0 < 0.05	71 ±7.6 0.50	51.3 ±11.0 0.10

TPR - Total peripheral resistance LVWI Left ventricular work index LVSWI - Left ventricular

dilution using Coomassie Blue and flushed peripheral injections as described in detail earlier (4 5)

Novadral at a concentration of 10 % in saline was infused into a peripheral vein with the aid of a Sigma pump equipped with an adjustable speedometer (Sigma motor Inc Middleport New York) The exact amount of Novadral infused per minute was measured after completion of the procedure by infusing it with the pump into a pipetting bottle Two speeds of infusion were used The first termed slow infusion , was adjusted to raise the systolic blood pressure by about 10—20 % The second continuing on from the first was a rapid infusion and raised the blood pressure about 20—30 % over the resting value The concentrations of Novadral per minute in the two infusions were as follows slow infusion 0 14—0 26 mean 0 17 mg/min rapid infusion 0 31—0 46 mean 0 34 mg/min

After the insertions of the needles a resting interval of about 20 min was allowed before starting the infusions The blood pressure and cardiac output were determined before infusion during the infusions and 5 10 20 and 40 min after cessation of the infusions

S gnificance refers to the change from the start ng values

Card ac ndex (l/ m n/m²)	TPR (un s)	LVWI (kg/ m n/m²)	LVSWI (g/m²)	Central blood vol (l/m²)	Tens on t me ndex (mm Hg sec/m n)	Effic ency (un ts)
3 25	14 7	3 9	53 3	1 25	2 761	1 42
±0 16	±1 1	±0 8	± 9 9	±0 13	±522	±0 28
2 53	22 5	3 5	57 5	1 13	2 664	1 31
±0 16	±6 6	±0 9	±14 5	±0 32	±358	±0 23
0 0	0 05	0 10	0 30	>0 30	0 30	>0 20
2 47	27 3	3 8	70 1	1 21	2 613	1 47
±0 17	±9 7	±0 8	±16 5	±0 18	±333	±0 39
<0 02	0 05	>0 50	0 0	0 0	>0 30	0 60
3 10	18 2	4 4	68 0	1 29	3 037	1 49
±0 19	±5 7	±0 8	±12 9	±0 15	±461	±0 30
0 60	0 05	0 05	0 01	0 80	0 30	>0 30
3 60	15 7	4 9	69 2	1 31	3 063	1 61
±0 87	±4 6	±1 06	±18 5	±0 30	±434	±0 44
0 10	0 30	0 0	0 05	0 80	0 10	0 90
3 72	14 4	4 9	67 7	1 26	2 882	1 73
0 78	3 8	±0 91	15 9	0 36	±344	±0 39
0 05	0 90	0 05	0 05	0 40	0 30	0 05
3 9	15 3	4 8	68 0	1 36	3 038	1 57
±0 82	±4 7	1 01	±17 5	±0 40	±423	±0 45
0 10	0 30	0 05	0 05	0 10	0 10	>0 90

s roke wo k ndex

Total per pheral res stance (TPR was calcula ed n absolute un ts by tak ng the ra o of mean brach al arterial blood pressure to CO n l n n The left ventr cular work ndex (LVWI and left entricular stroke work ndex LVSWI were obta ned by mul plying the correspond ng flow ndexes b 13 6 n ean brach al arter al press re 1000 The mean systol c blood pressure for calculat on of tl e tens on t me ndex TTI of Sarnoff et al 12 was obta ned by plan metry Eff c ency of the l eart was est mated by LVWI × 1000 TTI

Test ng of s gn f cance was done by pa red comparison and the t test

Results

The changes in the mean values are collated n table I and illustrated by figs 1—3 The s gn ficance refers to the change from the starting values

Blood pressure The induced rise of the systolic blood pressure was paralleled by a sign ficant elevation of both the diastolic and the mean pressure During the two infus ons the diastol c pressure rose from 74 to 84 and 93 mm Hg re spectively The correspond ng changes

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10 min after infusion	142 ±12.6 <0.05	79 ±6.8 <0.05	100 ±6.7 0.05	71 ±5.6 <0.80	50.9 ±13.6 <0.10
20 min after infusion	139 ±10.0 0.10	77 ±3.5 0.05	97 ±7.7 0.05	72 ±7.6 <0.80	52.0 ±17.7 0.10
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After the insertions of the needles a resting interval of about 20 min was allowed before starting the infusions The blood pressure and cardiac output were determined before infusion during the infusions and 5, 10 20 and 40 min after cessation of the infusions

after However, the determinations made 10 and 20 minutes after the infusions revealed significantly increased left ventricular pressure work

Left ventricular stroke work index showed a behavior similar to that of the total left ventricular pressure work, except that the rapid infusion evoked a significant increase This increase persisted as long as in LVWI

No significant changes occurred in the *central blood volume, tension time index* and *efficiency ratio*

Discussion

One of the clearcut changes evoked by Novadral was a strong reduction in the heart rate In all probability it was caused by enhanced vagal activity due to suddenly increased resistance to ejection, since this bradycardia was abolished already 5 minutes after cessation of the infusions, when circulating Novadral certainly still was present A corollary to this line of reasoning is the finding of Gredner and Neugebauer (1) who prevented the bradycardia with atropine

The principal site of action of Novadral was evidently the peripheral vascular bed which reacted by strongly reducing its cross-sectional area The mean increment in resistance was almost 100 per cent This makes the drug especially effective in hypotensive states due to deficient peripheral vasomotion On the other hand the strong bradycardia combined with lack of inotropic effect reduced the total blood flow considerably This feature was reflected in the unchanged left ventricular pressure work during the infusions in spite of the increased blood pressure The pressure work, however, was increased after cessation of the infusions, when the bradycardia was abolished and the blood pressure was elevated due to the summation of slightly elevated peripheral resistance and cardiac output

A slow heart rate can be beneficial in giving more time for diastolic filling and in reducing the oxygen requirements of the heart (7) The latter feature was illustrated by the unchanged tension time index despite the increased blood pressure Nevertheless, the heart was not made more effective because the pressure work and the tension time index moved in the same direction, resulting in an unchanged efficiency ratio

Apparently no major shifts occurred in the distribution of the circulating blood, so far as can be deduced from the unchanged central blood volume

With regard to cardiogenic shock, in which the peripheral resistance is seldom primarily insufficient but in which the fault lies in a reduced total blood flow due to inadequate stroke output, a pressor amine with inotropic properties is to be preferred This causal therapy, in the hemodynamic sense is indicated even in the face of possible rhythm disturbances evoked by the drug Against this background the use of Novadral in cardiogenic shock is less rational In hypotensive states due to inadequate peripheral vasoconstriction, including certain cases of myocardial infarction especially in a febrile phase (10) the use of Novadral is indicated

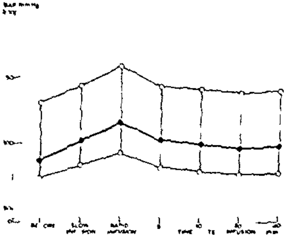


Fig. 1. The brachial arterial pressure before, during and after Novadral *infusions*.

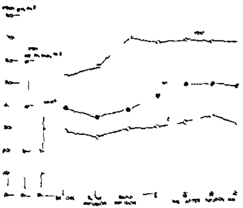


Fig. 3. Changes in left ventricular stroke work index (LVSWI), left ventricular work index (LVWI) and efficiency evoked by Novadral.

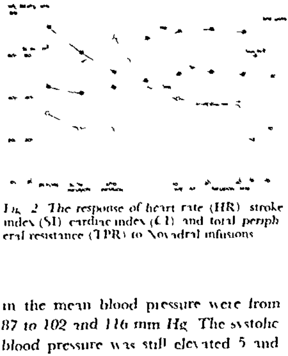


Fig. 2. The response of heart rate (HR), stroke index (SI), cardiac index (CI) and total *peripheral* resistance (TPR) to Novadral infusions.

in the mean blood pressure were from *87 to 102* and *116 mm Hg*. The systolic *blood* pressure was still elevated 5 and 10 minutes after discontinuation of the infusion. The rise in diastolic and mean pressures persisted up to 20 minutes after the infusion.

Heart rate. A strong bradycardia developed during the infusion. The first infusion reduced the heart rate by 12 beats/min. on the average, and the following rapid infusion further reduced it by 9 beats/min. Determinations after cessation of the infusions revealed that the heart rate had reverted to the starting values already after 5 minutes.

Stroke index showed no consistent pattern during the infusions. The slow infusion reduced it in 4 cases and increased it in 2 cases, in 4 cases it remained almost unaltered. The rapid infusion increased it in 3 cases, decreased it in 3 cases and caused no change in the remaining cases.

Cardiac index. A significant reduction occurred in this parameter, averaging 0.73 l/min/m² during the slow infusion. The rapid infusion evoked a further slight reduction. The total decrement averaged 24 per cent from the starting values.

Total peripheral resistance rose sharply from 14.7 to 22.5 units during the slow infusion and to a mean of 27.3 units during the accelerated infusion. It was still significantly elevated 5 minutes after the infusions, but thereafter it declined to the starting values.

Left ventricular work index was unmodified by the infusions and 5 minutes there-

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Haematemesis and Melaena from a Limited Reception Area During a 5-Year Period

By

Birger Herner Börje Källgård and Gunnar Lauritzen

The impetus to this study was a general impression that in the last decade haematemesis and melaena have become more common in the aged instead of as previously, having been more usual in middle age

Material

The 5-year material reported consists of patients treated at the Departments of Internal Medicine and Surgery of Varberg Hospital from 1957 to 1961 The hospital reception area comprises northern Halland with a population during the period of the study of 70 000 which is about 1 % of the population of Sweden The area has only this county hospital It should therefore provide a truer picture of the incidence than many earlier published data relating to areas without a centralized medical service The area contains two towns of 15 000 and 5 000 inhabitants representing nearly 30 % of the population The working population is engaged largely in agriculture and its subsidiary trades Of the total number of patients 63 % were from the countryside and 37 % from the towns

Submitted for publication October 12 1964

The evaluation of the material is uniform in that the same physicians were in charge of the medical surgical and radiological clinics during the entire period

Extent of the material and arrangement as to diagnoses

All cases of haematemesis and melaena were included while rectal haemorrhages were excluded Red blood on or in the stool was taken as rectal haemorrhage unless there was *manifestly* a severe gastrointestinal haemorrhage with rapid passage through the intestines At least two positive tests for blood in faeces were required for a diagnosis of melaena A reduction of the haemoglobin figure on the other hand was not a diagnostic requirement since even a relatively ample haemorrhage need not be reflected in a recordable reduction of haemoglobin

The material consists of 508 episodes in 422 persons The grouping by diagnoses is shown in table I

Since the study relates to peptic ulcer, the account will comprise in the sequel only cases of gastric, duodenal and gastrojejunal ulcer Cases of melaena of doubtful genesis have also been includ

Summary

The hemodynamic changes evoked by Novadral[1] were evaluated in 10 normal subjects by intravenous infusions Novadral caused a marked reduction in the heart rate and cardiac output and a prompt elevation of the peripheral resistance No inotropic effect could be demonstrated on the heart Myocardial oxygen consumption, as roughly reflected by the tension time index, paralleled the changes in the pressure work of the heart The use of Novadral is briefly discussed in the light of the hemodynamic findings

Acknowledgements

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50 per cent or below, comprises 113 episodes, while "Mild haemorrhage", cases in which the lowest Hb record was always above 50 per cent, comprises 309 episodes

One case is omitted in the table since the patient died before haemoglobin determination could be done

There have thus been nearly three times as many mild as severe haemorrhages which is a remarkably high incidence for the former Of the 113 episodes of severe haemorrhage 68 were in males and 45 in females

Haematemesis occurred in 39 8 per cent of the cases of severe haemorrhage and in 29 4 per cent of mild cases

TABLE II Breakdown by number of ulcer patients and number of admissions

No of admissions	No of patients	%	No of episodes
1	282	84 1	282
2	39	11 0	78
3	11	3 2	33
4	3	0 8	12
5	1	0 3	5
6	1	0 3	6
7	1	0 3	7
	338	100 0	423

TABLE III Breakdown by age and diagnosis

Age	Ulc ventr	Ulc. duod	Melaena	Total
<30	8	8	5	21
31 –40	5	13	13	31
41—50	27	38	12	77
51—60	22	42	19	83
61—70	33	38	19	90
71—80	42	30	13	85
>80	17	15	4	36
Total	154	184	85	423

Principles of treatment

Hospitalization Cases of haematemesis and melaena are so unpredictable and hazardous that there can be no doubt of the necessity for treating them in hospital If their general health is seriously affected being adjudged more generally critical than the Hb level would suggest, we have kept them in bed during the first days They have been allowed a reasonable mobility however though visits to the toilet under supervision and a short period of sitting up during making of their beds were usually granted only after a couple of days As a rule patients have not been allowed to be up for a lengthy period until after a negative test for blood in the stool, and they have not generally been discharged until after three negative tests The time in hospital has usually been between 2 and 3 weeks

Diet Seriously affected patients have been given a liquid diet during the first days As soon as they have emerged from the shock or preshock stage they have been put on a gastric diet The hospital has only one form of this diet, a liberal smooth diet in which substances are avoided which have a strong acid stimulating action on the gastric mucosa The gastric diet places some emphasis on milk foods and is as little mechanically irritative as possible It is heavily enriched with protein and vitamins

Medicamental therapy This has been liberal with antacids between meals and

TABLE I The 508 episodes with arrangement as to diagnoses

	Episodes	%
Ulcus ventriculi	140	27 4
Ulcus duodeni	184	36 1
Ulcus gastro-jejunale	14	2 8
Melaena causae incertae	85	16 7
Hernia hiatus oesophagi	3	0 6
Cancer ventriculi	54	10 8
Varices oesophagi	7	1 4
Alii causae (specified below)	21	4 2
Total	508	100
Neurinoma ventriculi	1	
Tumor intest ten	2	
Ruptura aneurysmae aortae c communicatione oesophagi	1	
Cancer coli	7	
Tumor malign c met hepatis	3	
Cancer pancreatis	4	
Cancer duct hepat	1	
Diverticulosis coli	2	
Total	21	

ed since, like many other authors, we consider that the cause in most of these conditions is ulcer *The material is thus reduced to 423 episodes in 338 patients, representing a haemorrhage incidence of about 1 2 °/₀₀ of the population of the area per annum* The subsequent discussion will be based on the number of episodes since, on recurrent haemorrhage, every patient constitutes a new diagnostic and therapeutic problem

Discussion of the material

It appears from table I that the ratio of duodenal to gastric ulcer episodes is 1 2 1, a remarkably high incidence of the latter To permit comparison with previously published data, gastrojejunal ulcers will be considered as gastric ulcers and deformation of the duodenal bulb as duodenal ulcer

Sex and age distribution

Of the 423 episodes 280 occurred in males (66 2 %) and 143 in females (33 8 %) Their distribution by age and diagnosis is shown in table III

The large number of elderly persons is striking Several of them were admitted repeatedly during the five year period In the 41—50 age group 18 % of the patients were in hospital more than once, while the figure for the 71—80 group is 30 % *Half of the patients were above 60 years of age* There is no great difference in the age distribution if one considers only the patient's age at the first haemorrhage during the 5 year period, in which case patients above 60 represent 47 7 % instead of 50 % for the entire material

Distribution according to severity of haemorrhage

In analysing haematemesis and melaena, the lowest haemoglobin value" is the only practicable criterion for division into mild and severe haemorrhage It is not free from objection, however, since there are several factors which cannot be evaluated afterwards, such as the patient s Hb at the start of bleeding, the speed at which blood was lost, and the speed and completeness of its compensation by blood transfusions The group Severe haemorrhage" in table IV, i e cases in which the lowest Hb record was

TABLE IV Comparison between London and Varberg (1964) materials in respect of gastrointestinal haemorrhage

Diagnosis	London (9) Episodes	%	Varberg Episodes	%
Ulcus ventriculi	423	16 7	140	27 4
Ulcus duodeni	844	33 4	184	36 1
X ray negative	816	32 3	85	16 7
Earlier operation	168	6 7	14	2 8
Hernia hiatus	47	1 8	3	0 6
Cancer ventriculi	57	2 4	54	10 8
Portal hypertension	75	2 9	7	1 4
Other causes	96	3 8	21	4 2
Total	2 526	100 0	508	100 0

therefore prove a valuable basis for other incidence studies

Diagnoses

There is no recent Swedish report which records all cases of haematemesis and melaena On the other hand there are several British and American studies which show the breakdown by diagnoses In a compilation of 5 192 cases of massive haemorrhage reported in the literature Gray et al (5) arrive at the following distribution peptic ulcer 65 % gastritis 11 % oesophageal varices 9 % hiatus hernia 2 % gastric cancer 1 % other causes 2 % and undetermined causes 10 The authors state that 15—20 % of haemorrhagic patients have negative X ray findings Another survey of 2 526 cases from the Central Middlesex Hospital in London during the years 1940—1957 is presented by Jones and Gummer (9) Their distribution of diagnoses is shown in table IV with the Varberg figures for comparison though with reservation for Jones definition of haemorrhage

Subject to this reservation one finds striking differences between the materials in respect of the incidence of gastric ulcer and gastric cancer The difference in number of X ray negative cases may be due to the varying requirements of the radiologist in respect of positive findings Many haemorrhages are considered to be caused by an acute mucosal lesion, which often heals within a few days and escapes detection by X ray These lesions like erosive gastritis can often be diagnosed only gastroscopically and should thus appear among X ray negative cases In our material we have attempted to perform an X ray examination as early as possible, while we have not employed gastroscopy

It may be of interest that a different method of diagnosis gives a different diagnostic spectrum Palmer (11) reports 650 cases of acute severe haemorrhage examined by the VDA method (vigorous diagnostic approach) i e gastroscopy and an X ray examination are made within one hour of admission In comparing the latter material with

also at night-time if the patient has had pronounced discomfort We have also used anticholinergics, usually in combination with sedatives, and an extra dose of vitamins B and C The latter have usually been given parenterally Iron, when administered, was in practically every case given orally No disadvantages have been noticed from this form of therapy

Blood transfusions These have been used principally for treatment of shock and preshock Grave haemorrhagic anaemia has also been treated with transfusions, especially in the aged

Surgical treatment Acute phase operations owing to continuous loss of blood with radiologically traceable source of haemorrhage have been done only in five cases Almost all cases with at least one or as a rule two serious episodes of haemorrhage have been operated on, however, though preferably not in the acute phase Consideration has been paid to the patient's age and to the X-ray findings, and each case has been assessed individually in consultation between surgeon and internist Owing to the high average age of the patients we have not always dared to operate to the extent that might have been desirable, since there were serious contraindications especially from circulatory organs and kidneys The typical operation has been gastric resection ad modum Billroth II Altogether 80 patients have been operated on (19 %)

Mortality

During the 5-year period 19 of 423 patients died owing to gastrointestinal haemorrhage caused by peptic ulcer or uncertain source of haemorrhage The mortality was thus 4 5 per cent, 10 men and 9 women, of whom 2 were aged 61—70 years, 12 aged 71—80, and 5 above 80 years

The diagnoses of the fatal cases were gastric ulcer in 13, duodenal ulcer in 3, melaena of unknown cause in 3 Fifteen of the deaths were in the medical department, four in the surgical Two of the latter had been operated on, but not during acute haemorrhage No patient died as a result of an acute phase operation

Comparisons between Varberg and previously published data

Incidence

The Varberg material reveals a comparatively high incidence of haemorrhage, 423 episodes in a population of 70,000 within a 5 year period, while Ward-McQuaid et al (16) report 400 cases among a British population of 200,000 in the same period and Sponheim (13) 144 cases in a Norwegian population of 125,000 in 2 1/2 years No definite conclusions can be drawn from these figures, however, since the materials are not compiled on uniform principles The danger of premature conclusions is also emphasized by Wenkert et al (17)

Thus no definite pronouncement can be made concerning the relative magnitude of the Varberg material But we would repeat that, because of its uniformity, it should provide a good idea of the absolute incidence of haemorrhage — about 1 per mille of the population within the area per annum — and may

2 5 1 Ihre and Muller (8) reported figures of 1 9 1 and 3 5 1 A common feature in all these studies is that they show strikingly numerous haemorrhagic gastric ulcers

Sex distribution

In the Varberg material two thirds of the episodes occurred in males, one third in females In the Stockholm study the ratio of male to female was 80 20 and in the Malmo 71 29 In Tomenius survey (15) of all ulcer patients in Stock holm from 1938 to 1952 the males numbered 70 % Ihre and Muller (8) found no differences between the sexes among patients with bleeding and non bleeding ulcers, the figure for men being 2 5 1 and for women 2 7 1

Age distribution

The age distribution of the Varberg ma terial differs considerably from earlier studies Half of the patients were above 60 years of age In the Stockholm series published by Aldman and Wallensten (2) 33 % were aged over 60 years Tomenius (15) material, comprising all ulcer patients, contained only 13 % above 60 years In a report on gastric haemorrhages from Mansfield Ward McQuaid et al (16) made a compilation from the literature of the number of bleeding ulcers above 60 years of age In the nine series studied by them the percentages varied between 34 and 50 per cent In a survey of bleeding ulcers from different decades Römcke (12) found that the number of patients above 60 years of age had risen continuously, from 21 % for 1935—40, 37 7 % for 1941—50, to 44 5 % for 1951—57

Haemorrhagic complication in the Varberg material is very much more common in elderly patients This has a serious effect on the prognosis Abramson (1), in a survey of ulcers in elderly persons from northern Sweden (Skellef tea) presumes that an increased arterio-sclerosis in the arteries of the stomach would be a contributory factor, though he finds no pathoanatomical evidence for this assertion Palmer (11) points out that the gastric arteries which are re sponsible for bleeding ulcers are very little susceptible to arteriosclerosis Bjornberg (3) states as reason for the definite increase of ulcers in the aged not only the growing length of life but also the general increase of the disease In his survey ulcer patients above 60 years of age numbered 10 % in the years 1912—40, 20 % in 1941—48, 35 % in 1949—54 The author explains this by the dif ficulty of obtaining a reliable anam nesis so that the lesions tend not to be discovered until a complication such as haemorrhage occurs Bjornberg also quotes a statement from 1946 by Boles et al to the effect that in autopsies of 97 ulcers in elderly persons only 10 had been diagnosed during the patient s life

Severity of haemorrhage

As already pointed out, the boundary between mild and severe haemorrhage has been put at a lowest Hb level of 50 % The same criterion was adopted by Aldman and Wallensten in their Stock holm material of bleeding ulcers and by Swedberg (14) in his report on gastro-intestinal haemorrhages from Marie-

TABLE V Distribution of haemorrhages by gastric ulcers, duodenal ulcers and melaena of doubtful cause in some Swedish materials

	Ulc ventr	(%)	Ulc. duodeni	(%)	Melaena caus. inc.	(%)
Varberg	154	37	184	43	85	20
Stockholm (2)	165	32	301	59	45	9
Malmö (17)	167	29	337	56	94	15

others, however, it must be noted that Palmer's series consists solely of severe cases of haemorrhage from the upper part of the gastrointestinal tract The result of the investigation by the VDA method showed 17 % oesophageal varices, 9 5 % erosive oesophagitis, 15 % similar lesions in the stomach, while gastric ulcers occurred only in 10 %, duodenal ulcers in 25 %, undetermined causes 13 5 %, and doubtful conditions 10 % About 45 % of the cases in his material were X ray negative A similar report from the Central Middlesex Hospital in London (4) showed 55 % of the haemorrhages to be caused by gastroduodenal ulcer, 40 % were considered to be due to an acute lesion, 2 % to cancer, and 3 % to portal hypertension

Differences in heredity and geographical environmental factors must also play a role in the genesis of intestinal haemorrhage In a paper from South Africa (6) dealing with 136 coloured patients, 25 had ulcers, 32 hepatic cirrhosis with oesophageal varices, while the most common causes were acute erosions and haemorrhagic gastritis Of those with definite ulcer disease some 70 % were below 40 years of age In a similar European study (e g table IV) ulcers occur in some 50—60 % of cases In a report from India the ratio of duodenal to gastric ulcers was 30 1 and of men to women 18 1 (9) The corresponding figures for Sweden according to Tomenius (15) are 2 4 1 and 2 5 1

Table V shows a comparison between the Varberg and two other purely Swedish studies But, as already noted, the values of such comparisons is limited by the varying character of the materials

The high incidence of gastric ulcers in the Varberg series may perhaps be explained by the comparatively high age of the patients This makes an X ray examination difficult and may result in an increased number of cases of doubtful melaena The ratio of bleeding duodenal to gastric ulcers is 1 2 1 in the Varberg material, 1 8 1 in the Stockholm, and 2 0 1 in the Malmo In Tomenius' (15) study of all ulcers — bleeding and non bleeding — in Stockholm the corresponding figure was 2 5 1 Malmros and Hiertonn (10) reported 687 cases of peptic ulcer in Örebro, of which 125 were haemorrhagic In the latter group the ratio of duodenal to gastric ulcers was 1 5 1 and in the entire material

in 338 patients representing a haemorrhage incidence of 1 2 per mille of the population of the area per annum The incidence of recurrences does not differ appreciably from the normal

The age of the patients was strikingly high 211 of the 243 (50 %) being 61 years or above and 121 (29 %) 71 years or above

The ratio of duodenal to gastric ulcers was 1 2 1 an unusually high incidence of bleeding gastric ulcers The sex distribution of two-thirds male and one third female accords with the tendency in other reports

There were about three times as many mild as severe cases if the borderline is put at 50 % Hb as minimum level recorded in an individual This comparatively high incidence of mild haemorrhages may be partly explained by the quick attention given to patients owing to the fairly concentrated reception area with good communications efficient coordination of out patient and in patient care and an actively regenerating therapy

Active regeneration as regards medical therapy consisted of liberal blood transfusions undogmatic dietary regime and early mobilization The average hospital stay was 2—3 weeks The principle in surgical treatment was to operate after one or usually two serious haemorrhages Operations were done in 19 per cent of cases the typical procedure being Billroth II gastric resection

The mortality was 19 cases out of 423 i e 4 5 % which is of the same order as in other reports Two of the fatal cases had been operated on but neither in the acute phase It should be *noted* that the material includes an unusual number of elderly persons and that no death occurred up to the age of 60 years, two being in the 61—70 age group and 17 in the over 70 age group

Compared with reports from elsewhere the Varberg incidence appears remarkably high But on account of lack of uniformity in compiling such surveys comparisons are unreliable and may lead to merely *apparent differences* The value of the Varberg material from the incidence aspect lies particularly in the fact that, through its uniformity, it should provide a comparatively good idea of the absolute incidence within the reception area, which is mainly a country district

The material may also be of interest, compared with other reports, when considering the *genesis of peptic ulcers* It is often assumed that the hectic pace of modern life is less prevalent in the countryside The stress factor that is presumed to be significant for the genesis of ulcers should in such case play a lesser role in our material which should be reflected in a comparatively lower incidence This is not confirmed, however, by our investigation For comparison an incidence study of a city population would be of great interest Apart from a medico-geographical perspective, it might be possible in due course to obtain an interesting time perspective For there is reason to suppose that within the next decades the population of the Varberg area will be very much more urban The occupations and localization of the population will change considerably as a result of the mechanization of agriculture and growing requirements of

TABLE VI. Mortality rate in different materials

	Ulc. ventr. (%)	Ulc. duodeni (%)	Melaena caus. ine (%)
Varberg	8.4	1.6	3.5
Stockholm	7.9	3.3	—
Malmo	12.6	2.9	—

stad. Severe haemorrhages were reported as 55 % in the Stockholm material and 27.9 % in the Mariestad. The corresponding figure in the Varberg material was 26.8 %. The reason for twice as many severe haemorrhages in the Stockholm report is difficult to explain, as also pointed out by Swedberg in his discussion of the Mariestad material.

Römcke (12), among others, states haematemesis to be a prognostically serious sign. In the Varberg series, on the other hand, haematemesis occurs roughly as often in mild as in severe haemorrhage. The same applies to the Stockholm material (2).

Mortality

The figures of mortality in bleeding ulcer vary greatly in the literature. According to Römcke (12), however, there has been definite decline in recent years from 5.3 % in 1935—40, 4.9 % in 1941—50, to 4.0 % in 1951—57.

But comparisons of mortality figures without taking into account, for instance, the patient's age may readily give misleading information. It is above all the mortality among younger patients with gastric haemorrhage that has definitely declined in recent decades. Römcke reports a decline of mortality for patients below 60 years of age from 4.3 % in the period 1935—40 to 0.3 % in 1951—57. In the Varberg material the total mortality was 4.5 % (19 deaths, all over 60 years of age) and in the Stockholm material 4.9 % (23 deaths with 4 below and 19 above 60 years).

The mortality among persons below 60 years of age in the Varberg material was zero and in the Stockholm material 1.1 %. Above 60 years the figures are 9 % and 11 %. In the Malmo material the total mortality was 5.4 %, for patients below 50 years of age 1.7 % and above 50 years 8.8 %. In a survey of nine British reports of bleeding ulcers the mortality varied between 4.5 and 9.3 %, being highest for gastric haemorrhages in every case. This is also confirmed by table VI.

Discussion and conclusions

The material consists of all cases of haematemesis and melaena treated at the medical and surgical clinics of Varberg Hospital during the 5 year period 1957—61. The hospital reception area has 70,000 inhabitants (about 1 per cent of the Swedish population), of whom some 70 per cent are resident in the countryside and 30 per cent in towns.

The total number of cases is 508, 63 per cent from country districts and 37 per cent from towns. To a large extent therefore, the patients are occupied in agriculture and its subsidiary trades. The presentation is concentrated to the number of cases of peptic ulcer, among which are included cases of doubtful melaena. There were 423 such episodes

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Sprue with a Characteristic Lesion of the Small Intestine Associated with Folic Acid Deficiency

By

W Veeger, O J ten Thije, N Hellemans, E Mandema, and H O Nieweg

The introduction by Dicke (5) of the gluten free diet has been a major advance in the treatment of celiac disease in childhood as well as in adults with sprue This regime however is not effective in tropical sprue according to reports dealing with patients from Hong Kong (6) and Puerto Rico (20) It also fails to relieve the signs and symptoms in some of the patients with non tropical sprue, therefore one may divide patients with non tropical sprue into two groups on the basis of their response to a gluten free diet (1) Cooke (3) and we ourselves (16) have also expressed the opinion that non tropical sprue is not a homogeneous entity Krevans (12) has suggested that non tropical sprue may be classified into two types using as the criterion the effect of folic acid on the intestinal manifestations

The present report deals with two cases of sprue associated with megaloblastic anemia in which treatment with folic acid produced not only a hematologic remission but also a disappearance of the intestinal symptoms and marked improvement of the abnormal morphologic aspect of the intestinal mucous membrane, even though these patients were not given a gluten free diet We believe that the changes observed in the jejunal biopsy specimen in these cases are characteristic

Material and methods

Two patients with sprue associated with megaloblastic anemia were investigated Ten patients with non tropical sprue without megaloblastic anemia who showed a good response to a gluten free diet and two patients with puerperal megaloblastic anemia due to folic acid deficiency without steatorrhea were also included in this study

The diagnosis in our patients was based on the history including a detailed nutritional history the roentgenologic examination of the intestinal tract and laboratory studies The response to treatment was evaluated during a period of clinical observation Patients with a history of a deficient diet were maintained in the hospital on a similar regime because we wanted to exclude the possible therapeutic effect of an adequate diet

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labour in industry (7) A future comparison between the present report and one from the same area after living conditions have undergone a radical and sociologically determinable change may provide valuable information concerning the significance of social environment for the genesis of the disease

Summary

During the 5 year period 1957—61 the Varberg Hospital treated 423 episodes of peptic ulcer accompanied by haematemesis or melaena This figure includes 85 cases of doubtful melaena The incidence of haemorrhages was 1 2 per mille per annum for the 70,000 inhabitants of the reception area who have access only to this hospital The age of the patients was strikingly high, half being 61 years or above, nearly one third 71 years or above The incidence of the disease was also strikingly high despite the fact that the reception area consists chiefly of a country district where stress factors should not be so common as in large cities The mortality was low Only 19 patients were lost, none below 60 years of age, only two between 61 and 70, and seventeen aged 71 years or above

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total epithelial area and number of nuclei was called the mean cell area. The area of individual nuclei was also measured with the planimeter on the photographs. Because the exact degree of shrinkage cannot be determined, we have expressed the measurements not in absolute values but in arbitrary units which may be used for comparative purposes. We could perform a large number of measurements on each photograph; therefore statistical evaluation was possible.

Case reports

Case 1. An intelligent white male aged 75, married, well-to-do, whose diet was entirely adequate. He left the Dutch East Indies in 1939, 22 years before admission.

In the tropics and afterwards he never had any complaints. Five months before admission he went to his general practitioner because of nausea, pyrosis and weight loss. Careful examination including hematologic studies and X-rays of the intestinal tract revealed only hypersegmentation of the granulocytes in the blood smear. Subsequently a period of constipation was followed by complaints about frequent voluminous grey stools. He also had anemic symptoms, slight jaundice and a sore tongue. Daily injection of 1 μg vitamin B_{12} over a 10-day period had no effect. After parenteral administration of 500 μg folic acid a day the steatorrhea improved and the blood picture showed a reticulocyte crisis of 22 % on the tenth day. There was no treatment with gluten free diet, corticosteroids or antibiotics. Twenty-one months after admission he was in good health, the stools and the blood picture were normal while his weight had increased 20 kg.

Case 2. A 46-year-old woman was first seen 7 years before admission with severe anorexia, vomiting after meals, heartburn, abdominal distension and weight loss. A gastric ulcer was suspected. Later on the possibility of anorexia nervosa was considered. Three months before admission slight anemia with an increased red cell diameter was found. After a period of constipation she developed diarrhea with grey stools. There was increasing weight loss and anorexia. She also had a sore tongue. After parenteral treatment with 500 μg folic acid daily the diarrhea disappeared and the blood picture improved with a reticulocyte crisis of 21 % on the fourth day. Before the folic acid administration vitamin B_{12} had been given without effect. It warrants mention that according to the hospital records of the first examination 7 years ago excessive fat was seen on microscopic investigation of the feces.

Results

The laboratory data of the two patients with sprue and megaloblastic anemia before and after treatment with folic acid are summarized in table 1.

On parenteral treatment with small doses of the folic acid the blood picture in both patients became normal. The steatorrhea disappeared nearly completely in the first case, while the second patient after 6 months passed stools of normal color and consistency once a day. In both subjects a continuous weight gain and subjective improvement occurred on this regime.

There was no treatment with gluten free diet, corticosteroids or antibiotics. Parenteral administration of 1 μg of vitamin B_{12} daily was ineffective as might be expected in view of the normal vitamin B_{12} levels of the serum. The folic acid activity of the serum of patient 2 was found to be low on two occasions (normal range 5—15 mμg/ml).

Jejunal biopsy specimens obtained from these two patients before treatment showed under the dissecting microscope a nearly flat mucous membrane. In the slides the associated total disappearance of the villi was seen. There was severe

The laboratory tests applied in the study of these patients included d-xylose test, glucose- and vitamin A tolerance tests and fat absorption the coefficient for which was determined by the van de Kamer technic (11) over a 5 day period on a daily fat intake of 80 or 100g. The vitamin B_{12} concentration of the serum was determined with the Lactobacillus Leichmannii method as described by Nieweg et al. (17), while the folic acid activity was estimated in the serum of case 2 with the Lactobacillus casei technic developed by Herbert (10). Vitamin B_{12} absorption was investigated with Schilling's urinary excretion technic (22). Urine was collected for 48 hours after oral administration of 1 μg Co^{60}-labeled vitamin B_{12}. In patients with megaloblastic anemia this test was performed after the completion of therapeutic trials with small doses first of parenterally administered vitamin B_{12} and subsequently of folic acid. Pancreatic function was investigated with the secretin test. Hematologic studies which included bone marrow puncture were performed with routine technics.

Jejunal biopsy specimens, obtained with the capsule of Crosby and Kugler (4) were studied before and after treatment, by means of a dissecting microscope and by light and electron microscopy. Immediately after the removal from the capsule the biopsy specimen was divided into 2 parts. One part was placed in a Petri dish containing 5 %, neutral formalin solution. The mucosal surface was inspected with a dissecting microscope (stereo microscope Wild M 5) and photographs of the mucosal surface of the specimen were taken. Technical details have been reported previously (24). Subsequently the tissue was dehydrated in alcohol, embedded in paraffin, perpendicularly sectioned in 5 μ sections and routinely stained with hematoxylin eosin and periodic acid Schiff.

The other part of the biopsy specimen was fixed in cold buffered (pH 7.35) isotonic OsO_4 solution. After 2 hours fixation the tissue was trimmed perpendicularly to the surface into small blocks of about 1 mm^3 with the aid of the dissecting microscope. These blocks were dehydrated and embedded in methacrylate for electron microscopy. From the same blocks sections were also made of thickness about 0.5 μ, with a Leitz ultra microtome and diamond knife for light microscopy. These sections were put on a slide floating on a drop of water, spread by heating and dried at room temperature. The metacrylate used for embedding was removed by dripping acetone on to the slide for about 5 minutes.

Staining was performed by a modification of the method given by Moore et al. (14). A saturated aqueous solution of crystal violet is heated on the microscope slide till it boils, excess dye is removed with running tap water, then a saturated aqueous solution of basic fuchsin is added and warmed till it boils and subsequently removed by rinsing with running tap water. The rest of the procedure consists of a short washing in distilled water, blotting with filter paper, drying at room temperature and mounting with neutral balsam.

Cross sectioned crypts at different heights were photographed in monochromatic light (Wavelength 5 550 Å) and an oil immersion Zeiss 100 x planapochromate. The small film negative was enlarged to 2,500 x. Photographs from specimens before and after treatment of the patient were taken on the same film and printed in one series.

In order to test the comparability of the enlargement of the photographs before and after treatment the greatest dimension of the nuclei of plasma cells was measured on the photographs. Statistically there was no significant difference between the size of the nuclei of plasma cells before and after treatment (test of Wilcoxon, $2\alpha = 0.05$). It is therefore unlikely that there are differences in the degree of shrinkage of the tissue specimens taken before and after treatment.

The total area of the cross sectioned crypts and of their lumen was measured on the photographs with a planimeter. The lumen area was subtracted from the total area. The number of epithelial nuclei including those of sporadic goblet and argentaffine cells was determined. Migrating lymphocytes in the epithelium were not counted. The quotient of

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There was no treatment with gluten free diet corticosteroids or antibiotics Parenteral administration of 1 µg of vitamin B_{12} daily was ineffective as might be expected in view of the normal vitamin B_{12} levels of the serum The folic acid activity of the serum of patient 2 was found to be low on two occasions (normal range 5—15 mµg ml)

Jejunal biopsy specimens obtained from these two patients before treatment showed under the dissecting microscope a nearly flat mucous membrane In the slides the associated total disappearance of the villi was seen There was severe

TABLE I Effect of folic acid administration on blood picture and intestinal function in two patients with sprue associated with folic acid deficiency

	Patient no 1			Patient no 2		
	Before treatment	After 6 weeks folic acid	After 22 months	Before treatment	After 4 weeks folic acid	After 6 months
Body weight (kg)	54 6	60 8	74 2	40	42	45 5
Hb (g/100 ml)	7 3	16 2	17 9	6 0	10 6	
RBC (mm^3)	1 50 10^6	4 35 10^6	5 15 10^6	1 56 10^6	3 52 10^6	
Serum iron (μg/100 ml)	244	96		196	25	
D xylose test (g/5 hrs)	0 9	3 3		5 6		
Vitamin A tolerance test (IU/100 ml)	65	150				
Folic acid activity (Mμg/ml)				0 8 1 5		
Vitamin B_{12} level (Mμg/ml)	0 97			0 68		
Schilling test (%/48 hrs)	0 7	15 1				
with intrinsic factor	1 4			29 5		
Fat absorption coeff (%)	68	84	Normal stools	79	77	Normal stools

damage of the surface epithelial cells with a narrow or absent brush border At electron-microscopic examination these brush borders consisted of shortened microvilli with wide interspaces At some places microvilli had entirely disappeared Massive migration of lympho cytes in the epithelium and increased in filtration of the stroma with plasma cells, lymphocytes and eosinophils were ob served

In addition to these abnormalities, which are generally present in untreated non-tropical sprue, we have observed a characteristic lesion of the jejunal epithelium which could be evaluated quantitatively in the 0 5 μ sections of the specimens from these two patients This lesion consisted of enlargement of the epithelial cells while the large irregular nuclei had a foamy appearance and enlarged nucleoli These features were seen in the crypts (fig 1) of the mucosa as well as in the surface layer of the epithelium (fig 2) The crypts showed a diminished number of mitoses and were infiltrated with lymphocytes

This characteristic epithelial pattern, the steatorrhea and the megaloblastic anemia disappeared when these patients had been treated with folic acid

After treatment there was remarkable regeneration of the jejunal mucous mem brane With the dissecting microscope a pattern of convoluted ridges in the first and leaf shaped villi in the second patient indicated that recovery of the villi was not fully completed when the biopsy was performed In the slides short and plump "villi" were seen The height of the "villi" was 1 x to 2 x that of the crypt layer The epithelium of surface (fig 3)

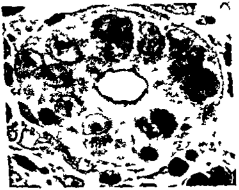


Fig 1 Transversely sect oned Lieberkuhn s crypt before treatment w th fol c acid megalocyt c ep hel um large w dely separated nuclei with enlarged nucleol Two m grating lymphocytes (0 μ sect on magn f cat on 1 600 x)

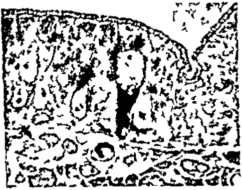


Fig 3 Surface ep thel um after treatment with fol c ac d normal epithel um w th brush border Goblet cell (0 5 μ sect on magnif cat on 1 600 x)



Fig 2 Surface ep thel um before treatment w h fol c ac d megalocyt c ep hel um w dely separated large nucle w h enlarged nucleol Brush border absent 0 5 μ sect on magn f cat on 1 600 x



Fig 4 Transversely sect oned L eberkuhn s crypt af er treatment w th folic acid normal ep thel um with goblet cells (0 5 μ sect on magn f ca t on 1 600 x)

and crypts (fig 4 had a normal appearance There was a normal striking m totic activity in the crypts At electron m croscopy the brush border of the sur face ep thelium consisted of normal slender m crovilli packed closely togeth er The length of the microvilli varied from 0 68—1 14 μ in the first and from 1 13 1 50 μ in the second patient M grating lymphocytes were seen only in the ep thel um at the top of the villi the stroma showed normal amounts of plasma cells lymphocytes and eosinophils Results of measurements of the areas of cells and nuclei before and after treatment with folic acid are given in table II

This pattern was present in only one of the untreated patients with non tropical sprue without megaloblastic anemia In this group of patients we observed the lesions also described by other authors

1 [illegible]

TABLE II A comparison of the mean cell and mean nuclear area in the jejunal epithelium before and after treatment with folic acid

Pat	Mean cell area		Mean nuclear area	
	Before	After	Before	After
1	552 (530—595)	243 (202—297)	137 (26—292)	80 (26—149)
2	567 (532—601)	235 (197—287)	166 (32—273)	81 (32—143)

(19, 27) There was a normal crypt epithelium, and lymphocytic infiltration was absent in that zone These 10 patients demonstrated the characteristic improvement on a gluten free diet (9, 13) The only patient in this group with enlargement of the intestinal epithelial cells and their nuclei had been admitted with bone pain due to osteomalacia 3 years ago when she had a typical response on a gluten free diet Biopsy was performed one year after she had discontinued this regime against medical advice At the time of biopsy the fat absorption was decreased There was no megaloblastic anemia and the Schilling test was normal The patient could not be studied more extensively due to circumstances beyond our control We have therefore no information about her nutritional status in respect to folic acid

In our two patients with megaloblastic anemia in the puerperium associated with deficient nutrition, a normal jejunal mucosal pattern was seen by the available technics Both patients, who showed no intestinal symptoms, had normal absorption studies while kept on their deficient diet They did not react to daily injections of 1 μg vitamin B_{12}, but parenteral administration of folic acid in doses of 200 resp 500 μg per day caused a reticulocyte response and their blood values returned to normal after treatment

Discussion

The characteristic enlargement of the cells, of the nuclei and of the nucleoli of the intestinal epithelium of our two patients with a sprue syndrome and megaloblastic anemia disappeared together with the anemia and diarrhea after the administration of folic acid This lesion may presumably be regarded as a manifestation of deficiency of this vitamin

Deficiency of a vitamin usually affects the tissues with the highest requirement of the vitamin in question In the case of folic acid the tissues primarily involved are those with the highest mitotic activity the bone marrow and the intestinal epithelium Folic acid is essential for the synthesis of DNA which occurs prior to mitotic division, because it promotes the formation of thymine and the purine bases through transfer of one carbon groups

In the bone marrow one may observe the characteristic megaloblastic pattern Similar changes — increase in size both of the cytoplasm and the nuclei with

enlargement of the nucleoli—were also observed in the small intestinal epithelium (at the surface as well as in the crypts) in the 2 patients with malabsorption due to folic acid deficiency This epithelial abnormality can be described as megalocytosis and is probably due to a disturbance in the production of the mucosal cells which occurs only in the crypts

The development of these cytologic changes in the small intestine is not surprising in view of previous studies showing an increase of the cell size in other non hemopoietic tissues This has been demonstrated by Graham and Rheault (8) in gastric epithelium and by Boen et al (2) in buccal epithelium from patients with untreated Addisonian pernicious anemia by van Niekerk (15) in cervical epithelium in megaloblastic anemia of pregnancy and by Gardner (7) in the buccal and gastric epithelium in tropical sprue

The cytologic changes induced by folic acid deficiency in the small intestine are however of greater clinical importance than those in other non hemopoietic tissues because of the resulting functional impairment a malabsorption syndrome

In puerperal folic acid deficiency one apparently may observe a megaloblastic bone marrow without lesions in the intestine One may assume that this is due to variations in the requirement of these organ systems under various conditions In the patients with megaloblastic anemia of the puerperium the rate of cell production in the bone marrow was perhaps increased in comparison with that in the intestinal crypts because of the increase of the red cell mass usually occurring in pregnancy and of the loss of blood at delivery

Vitamin B_{12} deficiency produces megaloblastic hemopoiesis similar to that induced by lack of folic acid Both these vitamins have been termed cytopoietic factors Because glossitis due to atrophy of the tongue epithelium and also megalocytosis of non hemopoietic tissues are features of both deficiencies it seems probable that vitamin B_{12} deficiency may also lead to megalocytosis of the intestinal mucosa and the associated functional impairment This may explain the excellent results of vitamin B_{12} administration observed in tropical sprue in Dutch military personnel returning from Indonesia (18)

We will now consider the factors causing the deficiency of the folic acid in our patients Folic acid deficiency in general may be due to an insufficient dietary intake, to an impaired absorption or to an increased requirement in the organism

Insufficient intake of folic acid seems an adequate explanation in our second patient with a 7-year history of abdominal complaints anorexia and vomiting At the first examination however sprue was not definitely excluded This patient has never been in a tropical area One may however assume that insufficient dietary intake was a major factor contributing to folic acid deficiency with a disturbance of cell production in the bone marrow and the small intestine resulting in megaloblastic anemia and sprue We have already mentioned that in puerperal megaloblastic anemia due to insufficient intake of folic acid the ab-

sence of intestinal megalocytosis is possibly due to a relative increase of mitotic activity of the bone marrow

In our first patient with sprue an insufficient dietary intake of folic acid could definitely be excluded Therefore there must have been an impaired absorption of this vitamin or an enhanced requirement or a combination of these mechanisms Even though this patient had never had any complaints in the tropics and for 22 years afterwards, we should like to point out the resemblance of the clinical picture of this patient to tropical sprue, for in this type of sprue glutens are well tolerated while good results are obtained by antibacterial treatment and folic acid administration The possibility of tropical sprue cannot be excluded in this patient

Perhaps a low-grade inflammation of the small intestinal wall due to invasion by bacteria or exposure to their toxic products causes an increased destruction and rapid regeneration of the intestinal epithelium, with an enhanced requirement for folic acid and some impairment of the assimilation of this vitamin Body stores of folic acid are known to be relatively small Once folic acid deficiency has developed the intestinal epithelium suffers further damage, with additional loss of function which establishes a vicious circle

In the tropics bacterial infections which, according to experienced observers often precede the development of sprue are far more common than in the temperate zone, but there is no obvious reason to suppose that this postulated pathogenetic mechanism should be active only in the tropics, though it will be less frequent in the temperate zone. It is clear, however, that an inadequate dietary intake of folic acid could promote the development of the sprue syndrome when intestinal infections are frequent (23)

The pattern of intestinal megalocytosis was seen only once in untreated gluten-induced non tropical sprue In this case folic acid deficiency could not be established on a firm basis because the patient withdrew from observation

One may regard a deficiency of folic acid and perhaps also of vitamin B_{12} as a distinct possibility in gluten induced non tropical sprue, because there is rapid regeneration of the epithelium and because the impaired absorption involves both these vitamins Reserves of folic acid, which is absorbed in the jejunum, are however exhausted within a period of weeks or months, while those of vitamin B_{12}, which is absorbed in the ileum, usually are sufficent for years (26) In gluten-induced sprue the intestinal lesion diminishes in intensity from proximal to distal (21) It is therefore not surprising that especially folic acid deficiency is not at all uncommon in this condition Folic acid administration does not, however, induce a remission of the intestinal symptoms in gluten induced non tropical sprue, even though it may improve megaloblastic anemia, because complete functional and morphologic restoration of the intestinal epithelium is impossible as long as exposure to gluten continues (25, 27)

In the two described patients with megalocytosis of the intestinal epithelium folic acid was the only factor needed for nearly complete recovery,

therefore we must assume a different trigger mechanism at the onset of this form of sprue

After these theoretical speculations we should like to point out the practical importance of the recognition of these patients in the temperate zones

This recognition may be facilitated by observing the megalocytosis of the epithelial cells in the small intestinal crypts At least some patients can be treated successfully with folic acid only and may not have to undergo the tedium of a long maintained gluten free diet

Summary

A characteristic abnormality of the intestinal epithelium was observed in biopsy specimens obtained from two patients with sprue associated with megaloblastic anemia Treatment with folic acid caused improvement of the anemia as well as of the intestinal lesion which is therefore considered to be a manifestation of folic acid deficiency This lesion which may be called intestinal megalocytosis was not found in 2 patients with puerperal megaloblastic anemia In 10 patients with gluten induced non tropical sprue it was observed only once The recognition of intestinal megalocytosis is believed to be of some practical value

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The Effect of Orally and Parenterally Administered Iron in Posthaemorrhagic Anaemia

By

Arne Foss Abrahamsen

Following moderate loss of blood the rate of blood regeneration is generally rapid even without treatment But after large protracted or repeated haemorrhages and in patients with small iron depots iron deficiency may become manifest (3)

For treatment of iron deficiency parenteral administration is generally practised only when oral administration for various reasons fails to give appropriate response Even in cases where such obstacles are not met with the parenteral route is claimed to be superior as judged from reticulocyte response (12) However the evidence presented to support this statement is weak, due to lack of adequate control groups

The present study deals with a comparison of the effect of orally and parenterally administered iron in patients suffering from posthaemorrhagic anaemia accompanied by low serum iron concentrations In addition an untreated group was included Irrespective of the route by which iron was administered nearly identical responses as to rise in haemoglobin reticulocyte number and serum iron were obtained In comparison, blood regeneration in the untreated group was poor

Submitted for publication October 16 1964

Material and methods

Thirty patients with recent or more chronic gastrointestinal bleeding were selected based on the following criteria 1 Initial haemoglobin concentration 10 g/100 ml or lower 2 Initial serum iron 50 μg/100 ml or lower Patients suffering from disorders known to influence erythropoiesis such as chronic infections renal diseases cancer or gastrectomy were excluded Most of the patients suffered from peptic ulcer with haemorrhage All patients were hospitalized and received the dietary regimen for peptic ulcer

The patients were allocated at random in one of three equally large groups One group was not given iron the other two groups were given iron by mouth or by parenteral route respectively

Oral administration of iron A daily dosage of 300 mg of iron as ferrous fumarate (Neo-Fer®) was administered in divided doses

Parenteral administration of iron 100 mg iron as iron-sorbitolcitric acid complex (Jecto-

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Results

The pre treatment values of haemoglobin and serum iron concentration were very similar in the three groups studied

Haemoglobin concentration

During treatment for 3 weeks the haemoglobin concentration rose by 4 8 g/100 ml and 4 7 g/100 ml in the two treated groups respectively (table I) In contrast, the untreated group showed a much lower haemoglobin rise 1 7 g/100 ml

Serum iron concentration

After treatment for two weeks serum iron rose to an average of 60 μg/100 ml in both iron treated groups (table II) Without iron therapy a fall in serum iron was generally observed (to mean 12 μg/100 ml) except in two patients with essentially unaltered values

Reticulocyte number

In iron treated patients maximum reticulocyte values occurred after treatment for 4—7 days Nearly identical values were seen in both groups (mean 260 000 mm³ and 250 000/mm³) In untreated patients the rise in reticulocyte number was poor (to mean 140 000 mm³) (table III) In this group a reticulocyte peak (mean 185 000 mm³) was observed in 5 patients when iron treatment was started at the end of the observation period

The reticulocyte response to iron treatment depends on the pre treatment haemoglobin concentration (12) This is clearly shown for both iron treated groups in fig 1

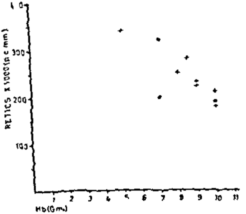


Fig 1 Relationship between initial haemoglobin concentration and peak reticulocyte count in absolute numbers
+ Oral treatment
● Parenteral treatment

Bleeding during treatment

Many patients bled during the first days of treatment However, the average duration of bleeding as judged from the benzidine reaction in the faeces was nearly identical in the three groups

In no instance did iron treatment have to be discontinued

Discussion

The present study has demonstrated that oral and parenteral administration of iron are equally effective in patients suffering from iron deficiency due to recent blood loss

Cope et al (4) in 1956 and Pritchard (8) in 1961 reached similar conclusions as far as gynaecological patients were concerned In other cases of iron deficiency anaemia Cope et al (4) found intravenously injected iron to be more effective However the selection of the patients receiving oral or intravenous

TABLE I Haemoglobin response to iron therapy

	Haemoglobin concentration (g/100 ml)		
	Untreated mean (range)	Oral iron mean (range)	Parenteral iron mean (range)
Initial	8 2 (4 5—10)	7 8 (5 5—10)	8 2 (5 0—10)
Increase in 3 weeks	1 7 (0 5—4 5)	4 8 (3 3—6 6)	4 7 (3 0—6 0)
Increase per week	0 6 (0 2—1 5)	1 6 (1 1—2 2)	1 6 (1 0—2 0)

TABLE II Serum iron in untreated and iron treated patients

	Serum iron (μg/100 ml)		
	Untreated mean (range)	Oral iron mean (range)	Parenteral iron mean (range)
Initial	29 (14—35)	38 (10—50)	28 (12—50)
After 2 weeks	12 (9—29)	60 (41—100)	60 (43—87)
Increase in 2 weeks	0	22 (5—63)	32 (14—52)
Decrease in 2 weeks	12 (3—22)		

TABLE III Reticulocyte response to iron therapy

	Reticulocytes (total numbers × 1 000/mm³)		
	Untreated mean (range)	Oral iron mean (range)	Parenteral iron mean (range)
Initial	64 (32—136)	80 (33 150)	63 (26 114)
Maximal response	140 (63—220(	260 (180 340)	250 (140 360)

fer®) was given intramuscularly once a day until the calculated amount of iron (minimum 1,000 mg) had been reached

Haemoglobin concentration was determined spectrophotometrically as cyanmethaemoglobin During the first 7 days daily analyses were carried out and later at least once a week

Serum iron concentration was assayed according to the method of Ramsay (9) Analyses were performed just prior to treatment and 14 days later Iron treatment was discontinued for 2 days before serum iron determination

Total reticulocyte count was determined daily for the first 10 days later once a week The reticulocytes were stained with brilliant cresyl blue using a modification of the Holboll method (10) (A total of 4 000 erythrocytes was counted in two different slides)

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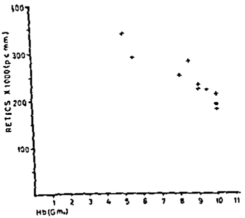


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iron is not well defined, as stressed by Beutler et al. (2).

Goetsch et al. (5) in *1946* found the parenteral route to be superior. They gave massive doses of colloidal iron (0.6—1.3 g) intravenously as a single infusion, and most of the patients developed severe toxic reactions. Due to lack of a control series, these authors compared the reticulocyte responses with those obtained by Heath in 1932 in an investigation with oral iron therapy. Thereby, several sources of error were introduced. As shown in this study, reticulocyte response to iron therapy is closely related to pre-treatment haemoglobin concentration. Further, *different techniques for* staining and enumeration of the reticulocytes may give very different results (1). In order to obtain reliable results, great care must be taken as to proper selection of the patients, as well as to relevant laboratory techniques. If such conditions are fulfilled, the therapeutic effect of orally administered iron seems to be as good as that of iron given parenterally.

The present study has shown that the blood regeneration was more rapid during *iron therapy than without such therapy*. The excessive low serum iron and low reticulocyte count at the end of the observation period, and a definite reticulocyte response when iron therapy was started, *strongly indicate an iron deficit* in the untreated patients.

The serum iron rose moderately during iron treatment, while it fell in the untreated group. The decrease in serum iron after an *acute loss of blood agrees* with previous experiences, and the lowest value is often reached after a week or longer (3, 6, 7, 11). Only occasionally is a transitory rise observed about one day after the haemorrhage (3, 6, 11). During regeneration of blood, the serum iron values remain at a low level, rising again *to normal only when the haemoglobin* has been essentially regenerated (6, 11). The low values for serum iron in untreated post-haemorrhagic anaemia reflect a disturbed balance in the internal iron metabolism (3). It seems rational, therefore, to continue iron treatment until the serum iron becomes normal, especially in patients who have had a more profuse or protracted haemorrhage. When iron absorption is not inhibited, equal results may be obtained by oral and parenteral *routes of administration*.

Summary

Thirty patients with recent gastrointestinal bleeding were allocated at random in three groups. One group was not treated, the two other groups were given oral or parenteral iron respectively. Blood regeneration was definitely more rapid during iron therapy. Nearly identical response with respect to rise in haemo*globin, serum iron* and absolute reticulocyte count was obtained irrespective of the route by which iron was administered.

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Thyro-cytotoxic Factor

By

Poul Halberg

Many features suggest that chronic thyroiditis (Hashimoto's disease) might have an auto-immune pathogenesis By now 3 different specific thyroid antibodies have been demonstrated in sera from patients with this disease, viz a complement fixing antibody (CFT) directed against an intracellular, microsomal antigen a precipitin (TRC) directed against thyroglobulin and an antibody directed against an antigen in the colloid which is not thyroglobulin (CA 2A) The question arises whether any of these serologically demonstrated antibodies actually have any effect on the thyroid cells proper or whether they should be considered passive phenomena of no physiological significance In 1959 Irvine (8) and Pulvertaft et al (12) demonstrated a complement requiring factor in sera from patients with chronic thyroiditis which was specifically toxic to thyroid cell cultures Later work (3 6 9 10) suggested that this cytotoxic factor (CTF) might be identical with the complement fixing antibody

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In this paper results are presented which bring further evidence that the cytotoxic factor and the complement-fixing antibody are identical The cytotoxic factor is demonstrated by use of cultures obtained by trypsinizing human thyroid tissue By means of an immune fluorescence technique and by autoradiography an attempt has been made to show that the cells thus obtained are follicular cells Thyroid cells which have been grown for 3 or 4 days develop resistance to the cytotoxic factor Experimental results which might explain this phenomenon are presented

Material and methods

Three hundred and sixty nine sera were examined Thirty-seven sera were collected from patients with histologically verified chronic thyroiditis Ninety-seven sera came from patients with toxic goitres all of whom underwent thyroidectomy in each case the diagnosis was clinically and histologically unequivocal Sixty-one sera came from patients who were thyroidectomized because of



Fig 1 Aggregates of cells obtained by trypsinizing thyroid tissue

atoxic goitres In all these cases the histological sections showed the goitres to be adenomatous without signs of thyroiditis or thyrotoxicosis Thirty four sera were collected from adult patients with non goitrous myxoedema In all cases subnormal values of PBI-127 and/or subnormal uptake of I^{131} were found Thirty eight sera came from patients with disseminated lupus erythematosus (L E D) and Sjogren s syndrome, and 51 control sera were drawn from random blood donors

The cytotoxic factor was demonstrated by means of cell cultures obtained by trypsinizing surgical specimens of toxic and atoxic goitres Ten per cent test serum and 10 per cent fresh normal human serum as a source of complement were added to each culture The procedure has been previously described (6) It differs little from the methods used by Irvine (8) and Pulvertaft et al (12) When reading the results allowance was made for variations in the sensitivity to the cytotoxic factor of cell populations derived from different thyroid glands The principle of this standardization has previously been described (6) In order to standardize the experiments further an attempt was made to use the same cell concentration in each experiment After trypsinization the cells were found in aggregates of unequal size (fig 1) Consequently exact cell counting was not possible So the cell concentration was determined by counting the amount of aggregates per 100 μl The average figure of 10 such countings was used as a measure of the cell concentration and the cell suspension was diluted until a concentration of 40 to 60 aggregates per 100 μl was obtained The method was checked by counting the nuclei according to the procedure described by Sanford et al (14) 40 to 60 aggregates corresponded to 3000 to 6000 nuclei Complement fixation reactions were performed as described by Roitt and Doniach (13) The specificity of all positive reactions was ensured by using a microsomal fraction as an antigen instead of a crude extract of thyroid tissue Microsomal fractions were prepared as described by Hogeboom and Schneider (5)

Thyroglobulin antibody was demonstrated by a passive haemagglutination technique using thyroglobulin coated sheep cells from Bourroughs Wellcome and Co (3)

CA 2 antibody was demonstrated by an immune fluorescence technique as described by Balfour et al (1) Since the results were sometimes blurred by high titres of thyroglobulin antibody sera containing thyroglobulin antibody in titres of 250 or more were excluded

Cell cultures intended for future treatment by the immune fluorescence technique were grown in ring chambers consisting of plexi glass rings glued on to slides by means of silicone grease The chambers were sterilized by flaming filled with cell suspension and closed by a cover slip which was also glued to the ring by means of silicone grease The cells would sediment and spread out on the slides Before staining the cover slips and the rings were removed and the cultures were carefully rinsed in saline fixed in acetone for 2 seconds and immediately after placed in a bath of barbitone buffered saline (Na barbitone 10 3 g 0 1 N HCl 40 3 ml NaCl 42 5 g distilled water to 5 l (1))

In this way the cells were adequately fixed without losing their antigenicity It was essential that the cultures never be allowed to dry during the whole procedure because the cells were fixed so lightly that otherwise they could easily be flushed off the slides The staining procedure was carried out as described by Coons and Caplan (2) The cells

TABLE I Occurrence of the cytotoxic factor (CTF) complement fixing antibody (CFT) thyroglobulin antibody (TRC) and antibody against second colloid antigen (CA—2A)

	No of sera	CTF	CFT	TRC	CA—2A
Chron thyroiditis	37	34	31	34	4 (of 6)
Myxoedema	34	23	15	29	12 (of 16)
Thyrotoxicos s	97	47	30	47	26 (of 62)
Atoxic adenomatous goitre	61	9	1	9	12 (of 55)
L E D and Sjogren s syndrome	38	5	4	19	18 (of 28)
Controls	51	4	0	10	7 (of 51)

were treated with a Hashimoto serum containing the complement fixing antibody in a titre of 1 024 for 60 minutes in a moist chamber at 37 C and washed in running buffer The slides were left in buffer for 30 minutes after which fluorescein-conjugated anti human globulin (horse origin) from Progressive Laboratories Inc U S was applied The conjugate was left for 30 minutes at room temperature after which the slides were rinsed again with running buffer and then left in buffer for 30 minutes After removal of the remaining silicone grease a drop of 50 glycerine buffer and a cover slip was placed on top of the cells Autoradiographs were also performed by means of cell cultures grown in ring chambers The cells were incubated with I¹ for 18 hours after which the cultures were rinsed with isotonic KI and fixed overnight in methanol saturated with KI The cultures were covered by liquid film Kodak Nuclear Track Emulsion Type NTB2 and stored in darkness at 4 C for 2 weeks

Results

Table I shows the occurrence of the cytotoxic factor in 318 sera from patients with chronic thyroiditis thyrotoxicosis myxoedema atoxic adenomatous goitres L E D Sjogren s syndrome and in 51 sera from blood donors The specificity of the cytotoxic factor was verified as mentioned previously (6) by incubating all positive sera with thyroid cultures which were not sensitive to the cytotoxic factor only if these cultures survived would the cytotoxic factor be considered specific In the control group 4 out of 51 sera were cytotoxic In 2 of the 4 cases the cytotoxicity was not specific The 2 specifically cytotoxic sera caused cytoplasmic fluorescence when the immune fluorescence technique was applied to unfixed ections of toxic thyroid tissue (7) whereas the 2 non specifically toxic sera were negative using this procedure In all other cases the cytotoxic factor was specific The sera were also examined for the occurrence of microsomal complement fixing thyroid antibody thyroglobulin antibody and Ca 2 antibody Table I shows the results Organ specific complement fixing antibodies (4) which occurred frequently in sera from patients with L.E D and Sjogren s syndrome are not tabulated

The group of sera from patients with atoxic adenomatous goitres differs little from the control group One serum from a patient with an atoxic goitre was markedly different from the others of this group This serum was cytotoxic,



Fig 1 Aggregates of cells obtained by trypsinizing thyroid tissue

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A similar experiment has been mentioned previously (6). No statistically significant correlation between sensitivity and antigenicity was found. In this experiment two changes were made. First an attempt was made to standardize the concentration of cells because the titre tends to go down if the cell concentration is particularly high. This phenomenon which was mentioned by Pulvertaft et al. (10) might be due to the fact that the cytotoxic factor is fixed to the dead cells allowing some cells to survive. Secondly the antigenicity of the extracts was determined by block titrations instead of titrations at one concentration only. The antigenicity was expressed as the product of the highest serum dilution and the highest dilution of extracts which still gave a positive reaction. In all cases the end point in the determination of the sensitivity of the cell cultures was well defined; experiments in which this was not the case were discarded. In this experiment the statistical correlation between the sensitivity of the cells and the complement fixing antigenicity of the corresponding extracts was significant (r = 0.70).

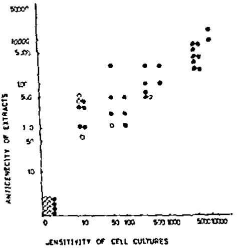


Fig. 5. Comparison between the sensitivity of cell populations obtained from 43 toxic (●) and atoxic goitres (○) and the complement fixing antigenicity of extracts of the corresponding thyroids.

Thirteen of the goitres examined were atoxic adenomatous. Four of these were both sensitive to the cytotoxic factor and antigenic in the complement fixing reaction. Nine were neither sensitive nor antigenic. Thirty three were toxic goitres. Four of these were neither sensitive nor antigenic; twenty nine were both sensitive and antigenic.

The sensitivity of the thyroid cells decreases during culture, and after 2 to 4 days the cells are entirely resistant, even to extremely potent sera (6, 9). In order to elucidate this phenomenon cells were grown in ring chambers, and at various times after the start of the experiment the cytoplasmic stainability by the immune fluorescence technique was tested in a number of cultures using a Hashimoto serum with complement fixing antibody in a titre of 1,024. Fig. 6 shows the results. All the pictures have been exposed and processed exactly the same way. The first picture (a) shows a cell culture which was stained 16 hours after the start of the experiment. A considerable cytoplasmic fluorescence is seen in all the cells. Cultures treated with normal sera revealed a very faint cytoplasmic fluorescence which could hardly be photographed. With a serum from a patient with L. E. D. as a negative control a pronounced nuclear fluorescence was seen, but hardly any cytoplasmic fluorescence. (b), (c) and (d) show cells which have been grown for 40 and 64

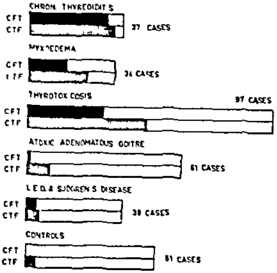


Fig. 2. Occurrence of the cytotoxic factor (CTF) and the complement fixing antibody (CFT) in 318 sera.

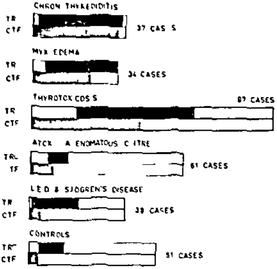


Fig. 3. Occurrence of the cytotoxic factor (CTF) and thyroglobulin antibody (TRC) in 318 sera.

contained complement fixing antibody in a titre of 32 and thyroglobulin antibody in a titre of 250,000. The histologic sections from this goitre were re-examined but no signs of thyroiditis or thyrotoxicosis could be found.

Figs. 2, 3 and 4 show the relative distributions of the cytotoxic factor, the complement-fixing antibody, the thyroglobulin antibody, and the CA-2 antibody. As previously found by Pulvertaft et al. (10), Irvine (9) and Halberg (6) in smaller materials, all sera containing the complement fixing antibody were cytotoxic. Some sera were cytotoxic without containing the complement fixing antibody, but none of the sera containing the complement fixing antibody were non-toxic. This finding indicates that the cytotoxic factor and the complement fixing antibody are identical, but that the cytotoxic factor is demonstrated by a more sensitive technique than the complement-fixing antibody. The incidences of the thyroglobulin antibody and the CA-2 antibody suggest that no connection exists between these antibodies and the cytotoxic factor.

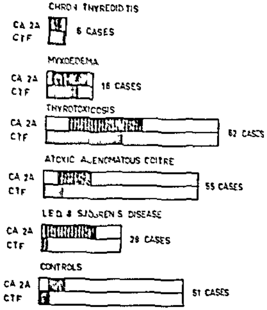


Fig. 4. Occurrence of the cytotoxic factor (CTF) and CA 2 A antibody in 218 sera.

Fig. 5 shows the correlation between the sensitivity of 45 cell populations to the cytotoxic factor and the complement fixing antigenicity of the corresponding extracts, the same standard serum being used throughout the whole experiment.

A similar experiment has been mentioned previously (6) No statistically significant correlation between sensitivity and antigenicity was found In this experiment two changes were made First an attempt was made to standardize the concentration of cells because the titre tends to go down if the cell concentration is particularly high This phenomenon which was mentioned by Pulvertaft et al (10) might be due to the fact that the cytotoxic factor is fixed to the dead cells allowing some cells to survive Secondly the antigenicity of the extracts was determined by block titrations instead of titrations at one concentration only The antigenicity was expressed as the product of the highest serum dilution and the highest dilution of extracts which still gave a positive reaction In all cases the end point in the determination of the sensitivity of the cell cultures was well defined experiments in which this was not the case were discarded In this experiment the statistical correlation between the sensitivity of the cells and the complement fixing antigenicity of the corresponding extracts was significant (r — 0 70)

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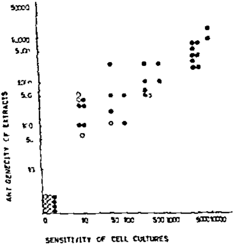


Fig 5 Comparison between the sensitivity of cell populations obtained from 43 toxic (●) and atoxic goitres (○) and the complement fixing antigenicity of extracts of the corresponding thyroids

order to elucidate this phenomenon cells were grown in ring chambers and at various times after the start of the experiment the cytoplasmic stainability by the immune fluorescence technique was tested in a number of cultures using a Hashimoto serum with complement fixing antibody in a titre of 1,024 Fig 6 shows the results All the pictures have been exposed and processed exactly the same way The first picture (a) shows a cell culture which was stained 16 hours after the start of the experiment A considerable cytoplasmic fluorescence is seen in all the cells Cultures treated with normal sera revealed a very faint cytoplasmic fluorescence which could hardly be photographed With a serum from a patient with L E D as a negative control, a pronounced nuclear fluorescence was seen but hardly any cytoplasmic fluorescence (b) (c) and (d) show cells which have been grown for 40 and 64

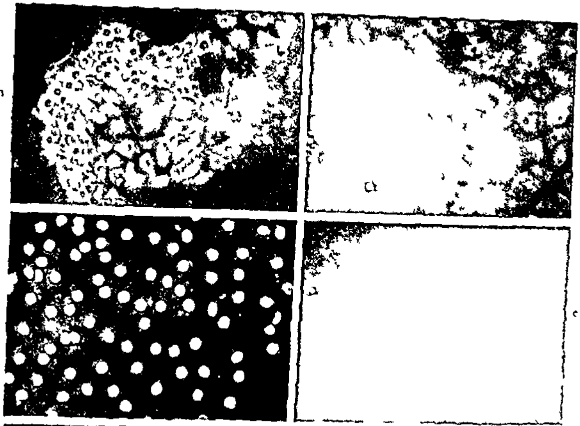




Fig. 6 Antigenic deletion of thyroid cell cultures a) 16 hour old cell culture treated by the fluorescence technique with a Hashimoto serum. Bright cytoplasmic fluorescence. b) 16 hour old culture treated by the same technique with a normal human serum. Nuclear fluorescence but no cytoplasmic fluorescence. c) d) & e) 40, 64 & 112 hour old cell cultures treated by the same technique with the same serum as in a. Cytoplasmic fluorescence intensity decreases

hours before staining The intensity of the cytoplasmic fluorescence is decreasing and the last picture (e) shows a culture which was grown for 112 hours before staining The cytoplasmic fluorescence is now extremely faint and it does not differ from the negative control With the assumptions that the cytoplasmic fluorescence represents the complement fixing antibody and that the complement fixing antibody and the cytotoxic factor are identical the result of this experiment would explain the decreasing cytotoxic effect of otherwise toxic sera when the cells have been grown for 2 to 4 days Apparently the cells lose the antigen corresponding to the cytotoxic factor during the first days of culture

The cells have been derived from thyroid glands and they look epitheloid (fig 7) but morphologic criteria are of

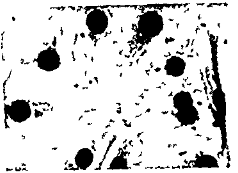


Fig 7 Thyroid cell culture Hematoxylin & eosin stain



Fig 8 Unfixed section of thyroid tissue treated by immune fluorescence technique with a Hashimoto serum Cytoplasmatic fluorescence of follicular epithelium

little value in tissue and cell cultures and it might reasonably be postulated that some or perhaps all of the cells are con nective tissue cells The fact that all the cells may be killed by cytotoxic sera seems to indicate that all the cells are identical and the fact that all the cells are stainable by the immune fluorescence technique with a Hashimoto serum in dicates that all the cells are identical with the follicular cells as seen in sections of a thyroid gland where the architecture of the tissue has been preserved, because these cells show fluorescence when treated in the same way (fig 8) In order to further elucidate the question of the identity of the cells autoradiography was performed on cells which had been incu bated with I^{131}



Fig 9 Autoradiogram of thyroid cell culture which has been incubated with I^{131}

Fig 9 shows an autoradiogram Un doubtedly the cells of this aggregate have taken up I^{131} which would indicate that the cells are thyroid follicular cells How ever the resolution is poor and some of the activity seems to be localized outside the cells Not all aggregates took up equal amounts of iodine and some of the aggregates did not take up any I^{131} at all Pulvertaft et al 10) suggested that each aggregate might represent one follicle If this is correct the ability of an aggregate to accumulate I^{131} might be an indication of the functional state of the correspond ing follicle

Discussion

It has previously been suggested that the cytotoxic factor and the complement fixing antibody are identical antibodies

directed against an intracellular antigen localized in the microsomes. This assumption was based on the relative distribution of these antibodies in various sera (3, 6, 9, 10) and on the fact that both the cytotoxic factor and the complement-fixing antibody could be absorbed by means of microsomal fractions of thyroid extracts (3, 6). Moreover it has previously been shown that if cells derived from a thyroid gland are sensitive to the cytotoxic factor, an extract made from the same thyroid would be antigenic in the complement-fixing reaction, and if the cells from a thyroid gland were not sensitive to the cytotoxic factor the corresponding extracts would not be antigenic (3, 6). An attempt to find a statistically significant correlation between the degree of sensitivity of the cells and the antigenicity of corresponding extracts was unsuccessful (6). The present results are in accordance with previous findings and further support the assumption that the cytotoxic factor and the complement-fixing antibody are identical. The respective distributions of the cytotoxic factor and the complement-fixing antibody in a series of 369 sera strongly suggest identity, and a new attempt to demonstrate a statistically significant correlation between cell sensitivity and complement-fixing antigenicity of the corresponding extracts was successful.

The incidences of the cytotoxic factor in sera from patients with chronic thyroiditis, toxic goitres and atoxic adenomatous goitres correspond well with those previously reported (6). The occurrence of the cytotoxic factor is considerably more frequent in the present group of sera from patients with myxoedema than in the corresponding group of the earlier paper. This difference comes in because a third of the sera mentioned previously came from patients with congenital myxoedema whereas all the sera mentioned in the present paper came from patients with adult myxoedema. The overall occurrence of the cytotoxic factor in the present material is less than in the materials presented by Irvine (9), Pulvertaft et al. (10) and Forbes et al. (3). This difference is probably due to the standardization of the results in the present and previous papers by the same author. In the two other papers no allowance was made for the variation in the sensitivity of cells derived from different thyroids.

The role of trypsinization of the cells in the manifestation of the cytotoxic effect has previously been discussed (6, 10). It might be assumed that trypsinization is necessary before the cytotoxic sera could be active, perhaps by making the cell membranes permeable for the cytotoxic factor. In that case the lack of effect of cytotoxic sera after 3 or 4 days of culture might be explained by a decreasing permeability of the cell membranes because the effect of the trypsin was wearing off. However, retrypsinization of 3- or 4-day old thyroid cultures does not make the cells sensitive to the cytotoxic factor again. On the contrary, retrypsinized cells will survive in the presence of even very strongly cytotoxic sera. By applying an immune fluorescence technique to the cell cultures as previously done by Forbes et al. (3) it could be demonstrated that the antigenicity of the cells disappeared in 4 days, which explains why the cells have then

become insensitive to the cytotoxic factor Apparently an antigenic deletion takes place during the first days of culture

Several facts seem to indicate hat the cells obtained by trypsinization and used for demonstrating the cytotoxic factor are pure thyroid follicular epithelial cells No cells survive the cytotoxic effect of specific sera, and all the cells are stainable by immune fluorescence technique with use of sera containing complement fixing thyroid antibody It can be demonstrated by autoradiography of cells incubated with radioactive iodine that the cells are able to take up iodine Pulvertaft et al (11) showed that cells obtained in a similar way and incubated with I^{131} were able to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) as shown by chromatography and Tong et al (15) who performed a similar experiment on cells obtained from sheep thyroids could make the cells produce not only MIT and DIT but also triiodothyronine and thyroxine

The mechanism by which chronic thyroiditis develops is still obscure and it is uncertain whether the antibodies demonstrable by serological techniques are really of any importance for the process Nevertheless one of these antibodies namely the complement fixing antibody does have a specific toxic effect on thyroid follicular cells, and consequently might be of pathogenic importance

Summary

Many features suggest that chronic thyroiditis (Hashimoto s disease) may be an auto-immune disease So far 3 different specific thyroid antibodies have been found Moreover, it is possible to demonstrate a specific cytotoxic factor active against thyroid cell cultures It has previously been suggested that this cytotoxic factor might be identical with one of the thyroid antibodies demonstrable serologically, namely a complement fixing antibody In this paper further evidence is presented that the cytotoxic factor and the complement fixing antibody are identical It is known that thyroid cells grown for more than 3 or 4 days are no longer sensitive to the cytotoxic factor In this paper it is shown by an immune fluorescence technique that during 3 or 4 days of culture an antigenic deletion takes place which might explain this observation Autoradiography of cell cultures incubated with I^{131} shows that cells are able to take up iodine This observation and the specific antigenicity of the cells demonstrated by the immune fluorescence technique, suggest that hey are thyroid follicular epithelial cells It is concluded that even though one of the circulating auto-antibodies found in chronic thyroiditis, namely the complement fixing antibody seems to have a specific cytotoxic effect on thyroid epithelial cells the pathogenetic role of these antibodies is still very uncertain

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Evidence Against "Bilirubin Sulphate"

By

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Bilirubin sulphate is regularly found mentioned in recent publications on bilirubin metabolism. A study of this literature, however, throws grave doubt upon the occurrence of this inorganic bilirubin conjugate (6).

Of similar hypothetical character is a synthetic bilirubin sulphate which Watson (8) obtained from bilirubin by exposing it to sulphuric acid and acetic anhydride. The product proved impossible to crystallize and quite labile and was believed to be a pyrryl sulphate in which the sulphate group was probably bound to a hydroxyl group of the bilirubin molecule. On paper chromatography it did not behave like a simple substance. It must be mentioned that the artefact was indeed alkali stable, i. e. no change occurred when it was submitted to the test of Billing et al. (1) in which 1 volume bile with 1/2 volume 0.1 normal NaOH is kept at room temperature in darkness for 30 minutes and then neutralized and analysed.

Isselbacher and McCarthy (5) pronounced in favour of the occurrence of bilirubin sulphate *in vivo* on the basis of an investigation which attracted much attention. After administration of $S^{35}O_4$ to rats, cats and human subjects, paper chromatography and autoradiography of the azo pigments of bilirubin from bile yielded results from which the occurrence of radioactive bilirubin sulphate was deduced. Since without presenting proof these authors regarded their "bilirubin sulphate" as identical with the alkali stable bilirubin pigment found by Billing et al. (1) in human bile, the physiologic occurrence of "bilirubin sulphate in the bile of animals and man" seemed evident to them.

Quite apart from the question whether a radioactive sulphate load in this case is a good method of gaining insight into the natural course of events, a few objections can be raised. For example, Isselbacher and McCarthy's bilirubin sulphate was not prepared in the pure state and then chemically identified. This is apparent in the following passage: "The analyses of human T tube bile for monoglucuronide and sulfate derivatives do

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not permit a conclusion as to the existence of a monosulfate or a disulfate derivative of bilirubin. It is possible that bilirubin conjugates containing both *glucuronic* acid and sulfate occur, as in the case of morphine". And to their statement that "One of these conjugates, *bilirubin sulfate, can readily be identified* in the bile of rats, cats and man after the injection of radioactive sulfate", we object that a chemical identification *has in* no way been made.

Schoenfield et al. (7) accepted the validity of Isselbacher and McCarthy's bilirubin sulphate and of their suggestion "*that the proportions of glucuronide* and sulfate conjugates might be altered in certain disease states". Moreover, in the experiments made by Schoenfield et al., rats were loaded with inorganic $S^{35}O_4$, *this time with or without preceding ex*perimental injury of the liver with the aid of CCl_4 or by ligation of the common duct. The conclusion was reached that this injury led to an increase in the bilirubin sulphate concentration. From a chemical point of view it must be pointed out, however, that these authors, like Isselbacher and McCarthy, omitted to present any *evidence* of the presence of bilirubin sulphate. We remain in the realm of hypothesis with the statement: "It has not been determined whether bilirubin sulfate exists as a monosulfate or disulfate or, indeed, whether a single bilirubin *molecule may be conjugated* with both sulfate and glucuronic acid".

Gregory and Watson (4) studied the bilirubin pigments in drain bile from dogs and human subjects injected with r*adioactive inorganic sulphate*. *Although* this treatment upset the natural conditions, they concluded that their experiments "have failed to demonstrate a *naturally occurring bilirubin sulfate in* human or dog bile". This negative finding is of interest also in view of the technique used. Working largely along the lines indicated by Isselbacher and McCarthy and by *Gregory* and Watson by further purification succeeded in establishing a complete separation between *the azobilirubin and the radioactive sul*phate — a result directly contradicting the findings of Isselbacher and McCarthy. Yet in conclusion they wrote: "Schoenfield and co-workers have amply *confirmed the finding of bilirubin sul*fate in the bile of the Wistar rat". And on the basis of personal observations concerning only a single rat injected with $S^{35}O_4$ Gregory and Watson concluded: "*These findings fully support* the conclusion that rat bile contains a bilirubin sulfate, but they are in striking contrast with the described results with human and dog bile". In this respect it may *be pointed out that Gregory* and Watson likewise omitted to prove the occurrence of bilirubin sulphate in the rat by isolation and chemical identification.

Finally, we may refer to a statement by Billing (2): "Isselbacher provides evidence that such a substance occurs in bile. It is unlikely that bilirubin sulphate normally accounts for more than 10 % *of the total bilirubin* excreted. This finding is in keeping with the observation that 10—15 % of the conjugated bilirubin in human fistula bile is not converted to bilirubin by mild alkali treatment." In this statement, again, the concepts of alkali stable bilirubin pigment and bili

rubin sulphate" threaten to be considered as identical without proof to this effect

Summarizing the salient points from the literature, we find that

1 "Bilirubin sulphate" has never been isolated as a chemical entity and analysed

2 It is generally assumed that "bilirubin sulphate" is alkali stable, unlike the alkali labile glucuronic acid ester of bilirubin (pigment II)

3 After treatment of bile with dilute alkali 10—15 % of the directly reacting bilirubin is encountered as alkali stable bilirubin pigment of a composition which has never been established

4 Without presentation of proof "bilirubin sulphate" is entirely or largely identified with the alkali stable bilirubin pigment of bile

Personal observations

In view of the great uncertainty surrounding the existence of bilirubin sulphate we have attempted to elicit facts which might warrant definite conclusions with regard to this hypothetical substance We considered it important to study human bile such as can be obtained in sufficient quantity from a common duct drain in adults So as to be quite certain that we were studying the composition of natural bile we deliberately refrained from injecting radioactive or other compounds

A basic consideration in this study was that in view of the alleged indentity of bilirubin sulphate and alkali stable pigment this sulphate must be localized in the alkali stable fraction formed when bile is exposed to the action of dilute alkali The first point to be established was whether the alkali stable fraction yields sulphate ion after acid hydrolysis if such a sulphuric acid ester is heated for 10 minutes to 100 C in 1 N HCl hydrolysis occurs in which SO_4 ion is formed) If so we should have to attempt isolation and identification of the sulphate bearing compound or compounds

If on the other hand, no SO_4 ion can be demonstrated after hydrolysis then the conclusion is justified that the alkali stable pigment is not a sulphate ester and therefore contains no bilirubin sulphate As we know SO_4 ion can be demonstrated reliably in very small quantities by the reaction with Ba ion or benzidine In calculations we reckoned on the most unfavourable possibility that *one* molecule bilirubin sulphate contains only one sulphate group It would then be possible by acid hydrolysis to split off one sulphate group per molecule "bilirubin sulphate"

Method

To 100 ml fresh human drain bile preferably ice-chilled and collected in darkness 150 ml 0 1 N NaOH is added After keeping this bile in darkness at room temperature for 30 minutes we acidify to pH 2 with about 4 ml 6 N HCl and then shake well Next 180 ml chloroform is added after renewed shaking we centrifuge for 3 minutes at 4 500 rpm The water layer and an intermediate layer are removed and the chloroform is freed of inorganic sulphate by washing at least twice with 100—150 ml 0 1 N HCl The chloroform solution is divided into two equal portions (each about 80 ml) In the first portion the alkali-stable bilirubin pigment and hydrolysable sulphate are determined without preceding purification the second portion is submitted to purification with an ion exchanger before these determinations are made The object of purification is to remove as much of the hydrolysable sulphate as possible while retaining as much of the alkali stable bilirubin pigment as possible The result of this purification can show that the two components occur independently and demonstrate that quite considerable amounts of ester sulphate unconnected with bilirubin can occur in bile (see no 12 table I) Purification is effected by treating the chloroform solution in question for 30 minutes with 4 g weakly active anion

TABLE I Comparison of the weight (mg) of $BaSO_4$ equivalent to the alkali stable bilirubin pigment of human drain bile with that obtained by acid hydrolysis of this pigment, both before and after purification with an anion exchanger

Drain bile no	Unpurified		Treated with ion exchanger	
	Calculated	Found	Calculated	Found
1	—	—	0.42	0.02
2	0.95	0.08	0.35	nil
3	0.92	0.11	0.65	0.03
4	1.7	0.19	1.4	0.17
5	1.3	0.27	1.0	0.11
6	1.5	0.24	1.06	0.15
7	0.77	0.16	0.63	0.15
8	0.85	0.45	0.63	0.02
9			0.42	0.03
10	0.85	1.15	0.68	0.36
11	0.27	0.78	0.28	0.17
12	0.28	0.48	0.05	0.21
13	1.10	1.15	0.71	0.43
14	0.80	0.37	0.60	0.25
15	0.92	0.17	0.22	0.11
16	0.40	0.94	0.34	0.23
17	0.44	0.22	0.38	0.09

exchanger (Amberlite IR45 (OH) CG type III) under constant stirring at room temperature and in darkness. After filtration (the filter is washed with about 6 ml chloroform) the filtrate volume is measured. On the fluid thus purified the above mentioned determinations are then made. The alkali stable (= directly reacting) bilirubin is determined by the method of Weber and Schalm (9). Since the alkali stable pigment is slower in reacting with the diazo reagent than bilirubin diglucuronide, extinction is measured after two hours.

For determination of the hydrolysable sulphate the chloroform solution is evaporated to dryness in a water bath. The residue is dissolved with the aid of about 6.5 ml distilled water and 1.5 ml 0.1 N NaOH and this solution is brought to about 1 N HCl with hydrochloric acid (12 N). Hydrolysis is then effected by heating in a boiling water bath during 90 minutes. The hydrolysate is allowed to cool and then neutralized with 5 N NaOH to approximately pH 2.5, it is then centrifuged and filtration is applied until a crystal clear solution is obtained. Addition of 0.3 ml 10 % $BaCl_2$ solution is followed by heating in a boiling water bath for 5 minutes, the solution is left standing for about 5 hours and then centrifuged. With distilled water the precipitate is transferred to a tube (8 mm) and washed once with 0.01 N HCl. After careful removal of the fluid we suspend the precipitate in 1 ml of a mixture of 1 volume glycerol and 1 volume water. The extinction of the suspension is measured at 470 mμ (cuvette 1 cm light path) in a Unicam SP 600 Spectrophotometer. In advance a standard curve is obtained by treating known quantities of $BaSO_4$ (prepared from Na_2SO_4 and $BaCl_2$) in an identical way. This standard curve is linear between 0.05 and 0.5 mg $BaSO_4$ per ml diluted glycerol. The quantity of $BaSO_4$ per ml of the suspension to be tested

s calcula ed bv mul pl ng the ext nct on found by the fa tor der ved f om the standard curve (n our case 1 15

Results

After a study of dra n b le from a number of adults v e found that as a rule even the unpur f ed chloroform solut on conta ned a cons derably lower concentrat on cf hydrolysal le sulphate than that pred c table from the concentrat on of alkal stable l l rub n p gment in th s solut on f the latter p gment v ere a b l rub n monosulphate W th one except on (no 12 analys s of the cl loroform solut on treated th on ex hanger y elded even lo er value as sl own n table I

Tl e only po s ble conclus on from l e e ob er at ons s tl at tl e alkal tal le l l ul n p gment ol ta ned from l uman d a n l le can conta n l ttle or nl e y small quant t es of ester sul pl a c e b l r l n sulpl ate Th s c n t tute a s rong argument aga nst the currence of l l rub n sulpl ate in l man l le at least n quant t es such as tl o e nd cated n the l terature

D a n b le o 3 and 5 was ol ta ned f m a female pat ent carry ng a dra n erted nto l e r gl t l epat c duct In l ca e tl e total b l rul n excret on tl t ok pla e a the r ght half of tl e l e l cl a h wn l y d appearance f the ja n f ce n tl s respect took o er l r f n of tl e wl ole l er In tl s l le or no apprec al le quant ty of l l t lpl e as demon trable In ew of th t mp bal le tl at forma of l l r l n s lpl ate co ld be an rt e ge c mecl an sm act ated hen e c l l l n excret o m st meet ex es e deman l

TABLE II Quant y of bil rub n d glucuron de and alkal stable b l rub n p gment found cteric serum

Ic er c serum no	B l rub n d glucu on de (mg/100 ml)	Alkal stable p gment (mg/100 ml)	%
1	8 7	5 2	60
2	7 3	3 6	49
3	20 6	10 9	53
4	7 5	4 9	65
5	2 6	1 7	66
6	5 8	4 3	75

Study of the format on of alkal stable pigment

Our attempts to collect fa ts argu ng aga nst the occurrence of b lirub n sul phate have not been confined to a s ngle route of approach We also made an in vest gat on nto the format on of alkal stable l lirub n p gment For f it could be shown that the p gment can occur under the nfluence of factors other than those operat ve n tl e format on of a sul phur c ac d ester one would be less n cl ned to ident fy the alkal stable p g ment with b l rub n sulphate

Tests u th te se un conta n ng onjugated b l rub n

When f esh cter c sera m is subm tted to tl e alkal stab l ty test of B ll ng et al 1) very h gh values are found for the alkal stable p gment determ ned ac cord ng to 9) as shown in table II It was ens red that in tl e e tests the pH l ad the same val e as in the pre iously descr bed tests with b le (pH about 12 3) Tl e alkal stal le p gment was al o dem onstrated by tl e column chromatograpl c method 3)

TABLE III Influence of normal serum, serum protein and deproteinized serum on the formation of alkali stable bilirubin pigment from bile

	Bil diglucur (mg/100 ml)	Alkali stable pigment (mg/100 ml)	n
Dry substance of			
2 ml bile + 2 ml H_2O	23 1	4 8	21
2 ml bile + 2 ml serum	22 1	9 1	41
2 ml bile + 2 ml protein fraction	22 6	8 2	36
2 ml bile + 2 ml deproteinized serum	22 6	4 8	21
3 vol bile + 1 vol water	16 9	3 4	20
3 vol bile + 1 vol serum	16 7	4 8	29
1 vol bile + 4 vol water	4 6	0 9	20
1 vol bile + 4 vol serum	4 4	1 7	39

These high values for icteric serum can hardly be ascribed to "bilirubin sulphate", for it has been adequately demonstrated that in this serum the pigment reacting directly with the diazo-reagent consists entirely or largely of bilirubin glucuronic acid ester

Tests with bile

Since it was suspected that in icteric serum the presence of protein might contribute to the formation of the alkali stable pigment a few experiments were carried out with bile to which was added normal human serum or the protein fraction prepared from it by precipitation with alcohol or acetone The results are presented in table III

These results show that the presence of normal serum or its protein fraction has caused a considerable increase in the quantity of alkali stable pigment Obviously this increase cannot have been caused by formation of bilirubin sulphuric acid ester, for the analysis was carried out immediately after addition of serum or protein Ester formation always requires a finite of time regardless of whether it is governed by enzymes or other catalysers The time interval in this test was insufficient for ester formation of any significance

TABLE IV Influence of normal serum on the formation of alkali stable bilirubin pigment from bilirubin diglucuronide (pigment II) obtained from bile

Pigment II	Bil diglucur (mg/100 ml)	Alkali stable pigment (mg/100 ml)	
In water	18 0	2 8	15
In serum	14 0	3 2	23

Tests with bilirubin diglucuronide (pigment II)

Pigment II was obtained from bile by means of column chromatography After elution with water the pigment solution was dried in a vacuum, and the residue was dissolved in water or in an equal

volume of normal serum The alkali stable pigment, obtained again at pH about 12 3 was determined in the same way as in the above mentioned tests The results are presented in table IV

Again, in the presence of normal serum the quantity of alkali stable pigment has increased, and again this cannot be ascribed to ester formation

In the above experiments, therefore, it has been impossible to explain the formation of alkali stable pigment on the basis of sulphuric acid ester formation This conclusion lends strong support to our view that this pigment can contain no (or only a trace of) bilirubin sulphate

What is the significance of these results for our understanding of bilirubin metabolism in adult man?

We have previously observed that the so-called bilirubin monoglucuronide or pigment I does not occur as a chemical entity in man but must be interpreted as a labile equimolecular complex of bilirubin and bilirubin diglucuronide (10) Since the occurrence of bilirubin sulphate in significant quantities in natural human bile is no longer plausible the scheme of the bilirubin metabolism in normal man has been reduced to its simplest form which merely signifies that the physiological metabolism of bilirubin takes place by conversion to the hydrophilic bilirubin diglucuronide As a secondary phenomenon a proportion of this conjugate can under certain conditions form with bilirubin a labile equimolecular complex which likewise is watersoluble Bilirubin sulphate plays no significant role in the bilirubin metabolism of adult man

We leave aside the significance to be attached to other bilirubin conjugates (e g bilirubin N glucuronide and the glucuronic acid ether of bilirubin) Such conjugates, which could occur only in subordinate quantities, have so far lived entirely hypothetical lives, like bilirubin sulphate'

Summary

A careful study of the literature showed that bilirubin sulphate has never been demonstrated as a separate chemical entity Without proof, it has been assumed that this substance is entirely or largely identical with the alkali stable pigment which can be obtained from bile

By means of acid hydrolysis of alkali stable pigment from normal human drain bile (during which bilirubin sulphate should have become split with sulphate ion formation) we found that this alkali stable pigment in general could have contained no or only trace quantities of bilirubin sulphate

We also obtained alkali stable pigment from icteric serum in quantities incompatible with a bilirubin sulphate composition The formation of alkali stable pigment from bile or pigment II under the influence of serum components demonstrated that bilirubin sulphate could not be involved

It is concluded that bilirubin sulphate plays no significant role in the bilirubin metabolism of adult man

Acknowledgement

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Book review

Therapeutic and diagnostic problems in severe renal failure By Nils Alwall 744 p 278 fig Munksgaard, Copenhagen, Svenska Bokforlaget Bonniers, Stockholm and Universitetsforlaget Oslo Bergen 1964

This new extensive monograph on the problems of renal failure is destined — according to the foreword — to fill a gap in the profuse literature on the artificial kidney and the pathophysiology of renal failure, due — in the author s opinion — to the fact that more attention was often paid to the theoretical aspects than to the many diagnostic and therapeutic problems connected with the use of the artificial kidney and the prolongation of life of these patients According to Alwall many hospitals are equipped with the artificial kidney but only a few of the clinical centres for dialysis function really well He sees possibilities of improvement in devoting much more care to all the details of the individual patient i e individual case histories

The latter actually make up the bulk of the book The first five short chapters deal with the principles of construction of the artificial kidney the composition of the dialysing solutions the equipment of a dialysing unit the Scribner shunt and they contain data on the development of treatment with the artificial kidney in Sweden reflecting the author's fight for recognition and against prejudice, crowned with eventual success

The 6th chapter, which the reviewer considers the most valuable, summarizes in 30 pages some of the diagnostic and therapeutic peculiarities which have so far attracted little attention outside of Lund Thus the necessity of feeding protein to chronically uraemic patients maintained on the artificial kidney is stressed Parenteral administration of glucose and fat emulsions in a concentrated solution by a plastic catheter into the vena cava is advocated Combating acidosis by inhibition of phosphate reabsorption from the intestine using aluminium and magnesium hydroxide is recommended Much space is devoted in this and the next chapter to the problem of fluid lungs' and to retroperitoneal oedema Neurological and psychological complications of these patients are discussed prophylactic dialysis is recommended for their prevention Good results in the treatment of the frequent severe lung infections have been obtained by a combination of antibiotics tracheotomy aspiration of mucous by bronchoscopy and the use of respirators in patients with respiratory failure Many asphyxtic patients owe their life to these measures Short notes are devoted to

urinary infection, intestinal paralysis (potassium depletion would deserve more space), to antibiotics which may accumulate and to the polyagglutinability of the erythrocytes Much weight is placed on early mobilization of the patient — important not only in combating the respiratory infection but also psychologically

Brief chapters are devoted to the use of the artificial kidney in the various intoxications, to the technique of ultrafiltration, and to assessment of the size of the kidney in the plain abdominal X-ray for diagnostic and prognostic purposes

The remaining 500 pages are case histories illustrating the different diagnostic and especially therapeutic aspects of acute and chronic renal failure They are documented by the diagrams which the students of these problems know from the numerous papers of the author and his school

It is certain that this monograph from the pen of a man whose name is so closely linked with the advance in this field (since Kolff worked out the first model of the artificial kidney 20 years ago), who has educated dozens of specialists now in charge of nephrological centres all over the world who has helped to build up many centres equipped with the artificial kidney in Sweden and elsewhere, and to whose untiring efforts hundreds and perhaps today thousands of patients owe their lives, will give rise to great interest and will be widely read There is no doubt that in his monograph Alwall has built a monument to the pioneering efforts in the field of the treatment of uraemia and to the immense contribution of his own group, which will always recall the many difficulties — medical, technical, ethical— which had to be overcome before the present status was achieved

Perhaps, in view of the popularity the book is likely to win, the author should have reconsidered whether the aim which he set himself, i e the clinical teaching of new adepts in the field of the artificial kidney, could not have been better achieved by extending the 6th chapter, by a more detailed analysis of electrolyte problems, especially potassium intoxication and depletion of renal function at least in cases when this is possible by a minimum urine flow, and by a reduction of case presentation to a few representative examples, analysed however in much more detail with discussion of every diagnostic and therapeutic decision and of every diagnostic and therapeutic error The reviewer feels that such a change in structure would greatly contribute to the readability of this valuable volume and should perhaps be considered when the second edition is being prepared

Jan Brod
Prague

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From the Medical Department VI, Sodersjukhuset, Stockholm, Sweden

Observations on Arterial Hemodynamics in Ventricular Extrasystoles

By

I G Porjé and B Rudewald

Different types of extrasystoles have been studied closely with the aid of electrocardiography We also know how the arterial pressure curves in different vessels are altered by extrasystoles Such studies indicate that a considerable disturbance of cardiodynamics is induced by extrasystoles However our knowledge about the hemodynamics is imperfect since the conventional methods for determination of blood flow in man have been inadequate for the study of rapid flow fluctuations The differential pressure technique worked out by us makes it possible to determine instantaneous values for blood velocity and flow in the proximal aorta In this paper some observations on the hemodynamics in the ascending aorta during a period of extrasystoles will be reported

Methods

The theoretical and experimental foundations for determinations of instantaneous aortic blood flow from a measured pressure gradient has been dealt with in earlier papers and only a brief description will be given here The present apparatus permits simultaneous recording of the aortic pressure and the differential pressure between two points along the ascending aorta As shown earlier (12, 13) integration of the differential pressure curve will give the wave form for the mean instantaneous blood velocity and flow between the measuring points To quantitate this wave form the distance separating the measuring points and the cross-sectional area of the aorta must be known These data can be obtained by radiological methods By further integration of the flow curve the volume of the blood flowing through the ascending aorta during one heart beat can be calculated This quantity corresponds to the left ventricular stroke volume minus the coronary flow during one heart cycle

Experimental procedure

To measure the differential pressure a double lumen catheter is introduced percutaneously into the arterial system Positioning of the catheter in the ascending aorta is performed under fluoroscopic control (fig 1) The double lumen catheter filled with heparinized saline is connected to a differential pressure transducer (fig 2) This instrument is an improved version of that described earlier by Porjé and Rudewald

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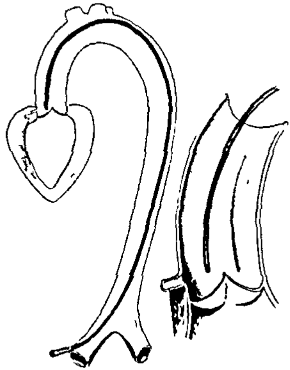


Fig 1 Diagram showing the position of the catheter in the aorta

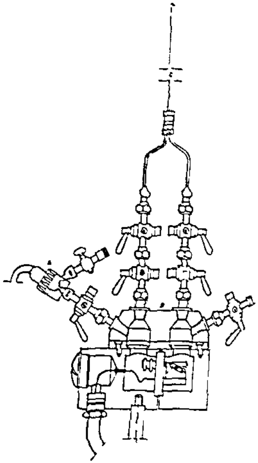


Fig 2 Catheter manometer system A Pressure-transducer, Statham P 23 G B. Differential manometer C Double-lumen catheter

(12, 13) A pressure transducer, Statham 23 G, is attached to one side of the differential pressure transducer This arrangement permits simultaneous recording of aortic pressure and differential pressure together with the ECG by the Elema four-channel ink recorder The result of dynamic calibration of the recording system is shown in fig 3 It will be seen that the resonant frequency is about 28 c/sec and the phase lag of the differential pressure transducer is less than 20 degrees for frequencies lower than 12 c/sec

In the patient referred to here diagnostic cardioangiography was performed using a biplane film changer designed by Gidlund (5) By direct measurements on the radiograms exposed in two perpendicular planes the cross sectional area of the aorta and the distance between the measuring points are determined Cardiac output was determined by the Fick method and by the isotope dilution technique according to Hedlund and Nylin

Results

A man, 42, with sub aortic and mitral valvular insufficiency Left ventricular angiography demonstrated sub aortic stenosis and mitral valvular insufficiency The aortic valve was normal and there was no dilatation of the ascending aorta The distance between the catheter side-holes was found to be 6 3 cm and the cross-sectional area of the aorta to be 8 1 cm² The latter value was obtained by averaging values during the period of extrasystoles

Cardiac output was found by the Fick method to be 5 8–6 5 l/min and

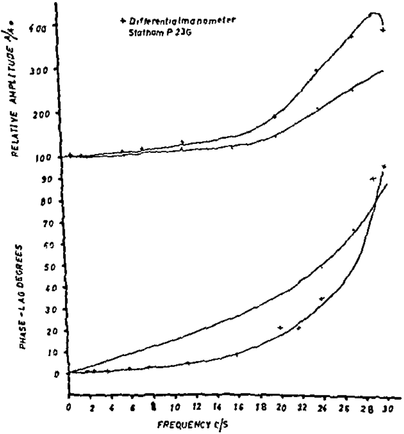


Fig 3 Amplitude response and phase lag of the transducer — amplifier — recorder system

the stroke volume to be 71—78 ml By the isotope dilution technique according to Hedlund and Nylin the cardiac output was found to be 6 1 l/min

ECG pressure and differential pressure curves recorded during the angiographic procedure are shown in fig 4 Graphical integration of the differential pressure curve is started at the onset of the last normal beat (marked 0) before the extrasystolic period and extended over the period to a normal beat The flow curves (IV) are obtained according to the principles outlined above The stroke volume calculated by the differential pressure technique compares well with that determined by the two other methods The injection of opaque medium into the left ventricle induces a series of irregular high frequency ventricular extrasystoles

In sinus rhythm there is a definite time relationship between the events of the ECG and the flow curve During ventricular extrasystoles an irregular pattern of flow pulses is observed with a varying lag with respect to the ECG From the cardioangiography examination it was

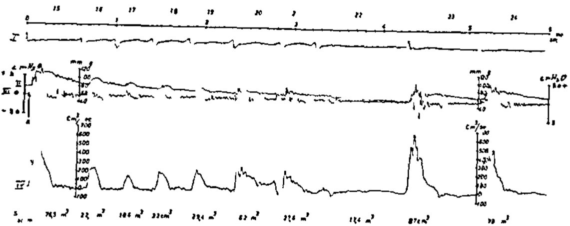


Fig 4 ECG, pressure and differential pressure curves recorded during the angiographic procedure

$$\text{Flow} = \frac{Q_m(t)}{L} \int_0^t \Delta p \, dt \text{ cm}^3/\text{sec}$$

L = Distance between pressure measuring points along the axis of the tube
Δp = Differential pressure between measuring points
$Q_m(t)$ = Mean cross sectional area of a portion of the ascending aorta between measuring points
I = ECG
II = Blood pressure just above the aortic valve
III = Δp curves
IV = Blood flow in the ascending aorta
O = Integration starts at this point

possible to study the movement of the aortic valve curves during the extrasystolic period It was observed that the aortic valve was closed during short intervals of time, corresponding to heart diastole During phases of valve closure a relatively high positive flow was recorded This is not the case in normal sinus rhythm Both mean pressure and pulse amplitude are reduced during the extrasystolic period The first 'normal" beat following the extrasystolic period has an increased stroke volume The blood pressure is re-established at its original level after a few beats

The first normal" beat after the extrasystolic period was observed to have an increased stroke volume This might be a consequence of Starling's law as it would be expected that the end-diastolic volume of the left ventricle should be increased However, such a mechanism has been doubted by Mejler et al (7) These authors consider the increased myocardial contraction as to be the result of post extrasystolic potentiation

Summary

The hemodynamics of the ascending aorta have been studied in a case where a series of high frequency ventricular extrasystoles were induced by injection of contrast medium (urografin 70 %) in the left ventricle The aortic blood flow was instantaneously determined by means of a special technique ECG, pressure and differential pressure curves from the ascending aorta were synchronously recorded These studies have shown that during the extrasystolic

period (4 3 sec) the flow in the ascending aorta is considerably reduced while the mean blood pressure and the pulse amplitude fall In some heart beats, relatively high positive flow is observed during diastole The first normal beat following the extrasystolic period is characterized by an increased stroke volume

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Body Build and Serum Lipids

By

Richard Hellstrom

There is convincing evidence of a relationship between body build and the concentration of cholesterol in plasma Thus, in 1936 Gildea et al (5) found higher levels in 17 men with pyknic body build, chosen according to Kretschmer s description than in 24 men of leptosomic physique In 1954 Gertler et al (4) showed that the proportion of mesomorphs (heavy muscles and large bones) was greater in a group of 97 men suffering from coronary heart disease than in a control group comprising 146 healthy men In the heart disease group moreover the plasma cholesterol level was higher for the mesomorphs than for the rest of the group In these investigations the determination of body build was based either on subjective judgement or on a number of body measurements that included complicated indices In the present study the body build was assessed by objective measurements namely the height of the subject and the distance between the outer surfaces of the femoral condyles The ratio of the former to the latter quantities has been referred to as the *skeletal quotient* (6)

Submitted for publication October 15 1964

Material

This material comprised 56 conscripts aged 19—20 years selected from a group of 178 conscripts studied in 1960 by Hellström (6) these had been drawn at random from some 900 conscripts They had been subjected to the same routine for the six months leading up to the investigation The 56 subjects were selected according to *the skeletal quotient* and *the height* which had been determined previously The group of 178 conscripts was divided into two parts according to whether the skeletal quotient was above or below the mean Each of these two groups was divided with respect to height — one group above the mean and the other below From these four groups the one with the highest and the one with the lowest values respectively for the skeletal quotient and the height were taken The 30 subjects with highest skeletal quotient in the former and the 30 with the lowest in the latter group were selected for this study These groups were labelled *the tall light* and *the short-heavy group* respectively Four of

TABLE I Anthropometric data in the two groups May 1960 Mean value ± S D

Group	No of subjects	Height (cm)	Femoral condyle width (cm)	Skeletal quotient	Weight (kg)	Skin fold thickness (mm)		
						Abdomen	Axilla	Scapula
Tall light	29	184 6± 3 4	9 76± 0 26	18 9± 0 36	68 9± 6 7	7 41± 3 64	5 72± 3 74	7 17± 3 54
Short heavy	27	171 7± 3 6	9 94± 0 29	17 3± 0 39	67 7± 7 6	9 63± 5 60	8 50± 6.97	9 30± 6 36
p		0 001	0 001	0 001	0 05	0 05	0 05	0 05

TABLE II Concentration of serum lipids and weight in the two groups Nov 1960 Mean value±S D

Group	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mmoles/l)	Weight (kg)
Tall light	169±24	201±34 3	0 86±0 35	70 9±6 1
Short heavy	188±30	204±40	0 88±0 42	68 5±7 9
p	0 02	0 05	0 05	0 05

these 60 conscripts were not available for the study

For the 56 remaining subjects samples of venous blood were withdrawn at 8 a m after an over-night fast At the time of sampling (November) the subjects had been in military service for 6 months in the same regiment

All subjects in the present study were personally interviewed with regard to coronary heart disease and gastric ulcer in their parents One subject in the short heavy group was an adopted child and had no knowledge of his parents When coronary heart disease was reported the hospital records were checked (2 cases) or the parents were examined personally (2 cases)

Methods

Within a few hours of collection the serum was separated from the blood by centrifugation The serum lipids were determined essentially as described by Carlson (2) Cholesterol was determined according to Sperry Webb, triglycerides according to Carlson (5) and phospholipids as phosphorus after combustion

Skin fold thickness and femoral condyle width was determined as described by Landegård (7, 8)

Results

The height and the skeletal quotient were greater for the tall-light than the short heavy group (table I) The condylar width was greater for the short heavy group There were no statistically significant differences in weight or skin-fold thickness

The concentration of cholesterol was significantly higher in the short heavy than in tall light group, but there was no

difference in weight or concentrations of phospholipids and triglycerides (table II)

The frequency of gastric ulcers reported among the parents in the two groups was of the same order (table III) Coronary heart disease was unknown among the parents of the tall light group, but was confirmed for parents of four of the short heavy subjects

In neither of the groups was there a significant correlation between cholesterol and skin fold thickness The tall light group increased significantly in weight during the six months of military service For another material (6) examined after the same period of service the skin fold thickness had decreased significantly

TABLE III Conscripts reporting the presence of coronary heart disease or gastric ulcer in a parent

	Coronary heart disease	Gastric ulcer
Tall light group $n=29$	0	12
Short heavy group $n=26$	[1]4	9

[1] 1 Father Myocardial infarction at age 52, characteristic ECG
2 Father Myocardial infarction at age 53 verified at autopsy
3 Mother Angina pectoris from the age of 55 ECG disclosed severe alterations
4 Mother Angina pectoris from the age of 50 ECG disclosed severe alterations

Discussion

Though the serum lipid level is known to be dependent on age and a number of external factors it is unlikely that these factors can account for the higher cholesterol level found in the short heavy group of the present material for the greatest difference in age was 2 years and all the conscripts had been exposed to the same factors such as diet exercise and daily routine for 6 months The findings suggest that persons with a short and heavy body build have a higher cholesterol level in blood than persons with a tall light build and that triglycerides and phospholipids are independent of the body build

Whether the incidence of coronary atheromatosis is higher among persons with a short and heavy stature than others remains to be proved Gertler et al (4) found a larger proportion of mesomorphs in a group of men suffering from coronary heart disease than in a control group Furthermore, Bjurulf (1) has recently found in a necropsy material that coronary atherosclerosis varied with body build, the shortest and most muscular persons having the most advanced coronary atherosclerosis The coronary heart disease found in parents of four of the short heavy group of the present study may have been due to atheromatosis which in turn may have its origin in an inherited disposition for a high cholesterol level in the blood

The determination of skeletal quotient is a simple method requiring only a measurement of the height and the femoral condyle width and calculation of the quotient of these two quantities, the procedure need take no more than a minute or so It provides an easy way of

classifying the body build The individual value can be compared with those for a representative standard material

Summary

The concentrations of cholesterol, triglycerides and phospholipids in plasma have been studied in two groups of conscripts, comprising 27 with the shortest and heaviest and 29 with the tallest and lightest body build, out of a material of 178 randomly drawn conscripts Any coronary heart disease and gastric ulcer among the parents was recorded

The concentration of cholesterol was significantly higher for the short heavy group than for the tall light group There was no difference between the groups as regards body weight, skinfold thickness and concentrations of phospholipids and triglycerides

Coronary heart disease was not found in any parent of the tall light group but in parents of four of the short-heavy group

Acknowledgement

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Artificial Ventilation with Tank Respirators in the Routine Treatment of Severe Pulmonary Failure Due to Chronic Pulmonary Disease

By

H GRENDAHL and H E REFSUM

Advanced chronic pulmonary disease may lead to increasing alveolar hypoventilation and severe arterial hypoxemia and hypercapnia Since severe oxygen lack may cause cellular damage and sudden death (12 13) there is frequently a definite need for relieving the hypoxemia However oxygen therapy is apt to be accompanied by further reduction of the alveolar ventilation and increasing carbon dioxide retention and in severe cases carbon dioxide narcosis may ensue The logical and safe therapy in such patients is therefore to apply artificial ventilation In some cases artificial ventilation makes it possible to increase the alveolar ventilation to such an extent that the arterial blood gases are completely normalized in others it may help to avoid further carbon dioxide retention and development of carbon dioxide narcosis while relieving a life threatening hypoxemia through administration of oxygen

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Since artificial ventilation of patients with pulmonary failure due to chronic lung disease was first described by Boutourline Young and Whittenberger (2) several reports have appeared which demonstrate the beneficial and frequently life saving effect of this treatment (1, 3 4 5 6 7, 17 18 19) Most reports deal with intermittent positive pressure breathing usually in combination with tracheotomy At Ullevål Hospital where equipment for intermittent positive pressure breathing as well as tank respirators have been available, artificial ventilation with tank respirators has been preferred as the standard method in this type of patients Semb et al (15) have reported on experiences with tank respirators in the treatment of pulmonary failure developing after thoracic operations and Refsum and Aarsvold (8) and Refsum (10 11) have presented detailed blood gas observations which demonstrate successful treatment of severe

TABLE I Patients with pulmonary tuberculosis and thoracoplasty treated with artificial ventilation

Patient No	Age	Sex	Factor leading to acute failure	Blood gas values prior to a v			
				CO_2 cont (mEq/1)	PCO_2 (mm Hg)	pH	HbO_2 (%)
1	35	♂	Post-operative	—	—	—	—
2	44	♀	Medicamental	37 2	92	7 21	54
3	39	♀	Pneumonia	[1]36 5	—	—	—
4	34	♂	Pneumonia	39 5	79	7 30	60
5	43	♂	Oxygen	[1]38 5	—	—	—
6	46	♂	Medicamental	39 5	71	7 35	68
7	61	♂	Oxygen	[1]33 9	—	—	—
8	37	♂	—	44 0	71	7 40	48
9	62	♂	—	34 6	69	7 30	48
10	57	♀	—	32 2	53	7 39	34
11	34	♀	—	34 0	65	7 32	80
12	58	♂	Pneumonia	40 3	76	7 33	44
13	54	♂	—	37 3	70	7 33	68
14	43	♂	Oxygen	34 1	61	7 35	43

[1] Venous sample

pulmonary failure in various types of patients with chronic lung disease

The purpose of this paper is to report on the results of routine treatment with artificial ventilation, in standard tank respirators, of patients with severe pulmonary failure due to chronic lung disease in the Department of Epidemiology, Ulleval Hospital in the period 1956—1963

Material

The series includes 32 patients with chronic pulmonary disease and acute pulmonary failure 27 men and 5 women The patients were admitted to the Department of Epidemiology from the Medical departments at Ullevål Hospital for artificial ventilation due to the arterial blood gas status (determined by H E Refsum), or the clinical picture of carbon dioxide narcosis, together with a high CO_2 content of the venous plasma In most patients the indication for respirator treatment was considered vital

Eighteen patients suffered from chronic bronchitis (table II), in the majority complicated with emphysema Fourteen suffered from pulmonary tuberculosis (table I) and had been treated with thoracoplasty, in 2 of these pneumonectomy had also been performed (cases 3 6) and in 1 segmental resection (case 1) On the average the operation had been performed 8 years prior to the respirator treatment The average age of the bronchitis group was 59 0 years (46—73 years), and of the thoracoplasty group 47 8 years (34—63 years) Twenty seven of the patients had clinical signs of heart failure, 22 showing signs of right ventricular hypertrophy either electrocardiographically or at autopsy

In 16 patients the acute respiratory failure developed gradually during several days without any apparent provoking cause the dominating symptoms being dyspnea, cyanosis, headache and drowsiness In the other patients the acute failure was possibly provok

(a v) in a tank respirator

State of consciousness at start of a v	Duration of a v until stabilisation of CO_2 cont (days)	Venous CO_2 cont when stabilised (mEq/l)	Survival time (months)	Cause of death
U	—	—	>138	
S	24	34 0	>102	
C	—	34 5	45	Resp failure
S	5	37 0	20	Tuberc empyema
U	4	30 0	69	Resp failure
U	9	31 0	46	Resp failure
U	11	31 5	33	
S	17	36 0	> 26	
S	16	30 5	1	Bronch carcinoma
S	9	30 0	2	Cardioresp failure
C	17	31 0	> 16	
S	45	34 0	11	Left heart failure
S	30	32 0	> 11	
S	4	27 5	> 11	

U = unconscious S semiconscious C conscious

ed by factors such as pneumonia oxygen administration or medicamental therapy depressing the ventilation opiates barbiturates) (tables I and II Three of the patients had received analgesics for a chronic headache probably due to pulmonary failure with carbon dioxide retention

Methods

Performance of artificial ventilation

Standard Emerson and Drinker tank respirators were used Seriously ill patients were ventilated continuously apart from short interruptions for nursing Oxygen was administered continuously by nasal catheter at a rate of 2—3 l min The patients were constantly supervised by an experienced nurse who ensured that the patient was following the rhythm of the respirator and that the airways were free

When a definite clinical improvement had taken place the duration of the artificial ventilation was reduced by 1—? hours daily When possible the tank respirator was gradually replaced by cuirass respirator The artificial ventilation was not ended until the clinical condition had been well improved and the CO_2 content of venous plasma had been stable for weeks

The frequency and pressures of the tank respirator were first set according to the spontaneous ventilatory frequency the body temperature the thoracic mobility and the severity of the carbon dioxide retention in the actual patient Later depending on the clinical condition or the changes in arterial blood gas values the respirator was readjusted The inspiratory and expiratory pressures were usually about 20—22 and 5 cm H_2O respectively and the frequency about 20 *per minute* Higher pressures are used at the present time

Analytical

The blood sampling and analytical procedures were performed as described previously (9 10) Arterial blood was drawn from the femoral artery and venous blood for deter

TABLE II. Patients with chronic bronchitis treated with artificial ventilation (a.v.) in a tank respirator

Patient No	Age	Sex	Factor leading to acute failure	Blood gas values prior to a.v. CO_2 cont. (mEq/l)	PCO_2 (mm Hg)	pH	HbO_2 (%)
15	62	♂	—	[1]35.5	—	—	—
16	52	♂	Pneumonia	36.0	65	7.35	63
17	55	♂	—	31.5	66	7.28	64
18	52	♂	Medicament & oxygen	[1]42.0	—	—	—
19	46	♂	Pneumonia	[1]41.5	—	—	—
20	57	♂	—	36.0	73	7.30	33
21	61	♂	—	43.2	92	7.28	38
22	62	♀	—	36.5	80	7.25	27
23	63	♂	—	36.5	60	7.39	81
24	65	♂	—	35.6	75	7.28	68
25	58	♂	—	40.6	95	7.23	27
26	54	♂	Medicament	37.0	73	7.31	51
27	65	♂	Pneumonia & oxygen	35.0	110	7.10	—
28	73	♂	—	39.2	—	—	41
29	64	♂	Pneumonia	34.0	68	7.30	49
30	54	♂	—	46.0	86	7.33	57
31	53	♂	—	[1]43.4	—	—	—
32	66	♂	Medicament & oxygen	35.3	95	7.17	41

[1] Venous sample.

mination of the venous plasma CO_2 content from the cubital vein, stasis being kept to a minimum. With limited facilities for routine determinations of the arterial blood gases, the adequacy of the artificial ventilation was evaluated by means of the venous plasma CO_2 content. Taking into account that the CO_2 content is usually about 2 mEq/l lower in arterial than in venous plasma, and that arterial pH during the recovery from hypercapnia is usually higher than 7.4, it follows that reduction of the venous CO_2 content to 30 mEq/l and lower in most cases indicates that the arterial CO_2 tension is normalized.

Results

Table I shows the observations in the patients with pulmonary tuberculosis and thoracoplasty, and table II the observations in the patients with chronic bronchitis. Of the 32 patients in the series 11 were unconscious and 16 semiconscious at the start of artificial ventilation.

Among the patients with pulmonary tuberculosis and thoracoplasty (table I) 4 were unconscious and 8 semiconscious at the start of artificial ventilation. All improved clinically and were able to answer questions after a few hours' treatment. Some patients, however, had periods of disorientation during the next 2—3 days. During the first 3 weeks after the start of artificial ventilation there were no deaths. The average duration of the artificial ventilation until stabilization of the venous plasma CO_2 content

State of consciousness at start of a.v	Duration of a.v until stabilisation of CO_2 cont (days)	Venous CO_2 cont when stabilised (mEq/l)	Survival time	Cause of death
C	90	33 5	24 m	Heart failure
S	—	—	4 d	Resp failure
S	—	—	10 m	Cardio resp failure
U	3	35 0	3 m	Cardio resp failure
S	15	32 0	>69 m	
S	18	32 0	4 m	Cardio resp failure
U	—	—	8 d	Pulmonary embolus
U	—	—	0	Resp failure
C	150	33 0	12 m	Carcinoma of stomach
U	25	28 0	25 m	Heart failure
U	—	—	0	Resp failure
U	17	29 5	>29 m	
U	7	29 0	>29 m	
S	—	—	11 d	Bleeding duod ulcer
S	—	—	2 d	Resp failure
C	3	35 0	>11 m	
S	20	36 5	8 m	
S	19	35 0	> 2 m	

U unconscious S—semiconscious C—conscious m=months d=days

was 16 days, the average CO_2 content then being 32 3 mEq l

Among the patients with chronic bronchitis (table II), 7 were unconscious and 8 semiconscious at the start of artificial ventilation Six patients died in the course of the first 3 weeks 2 patients (cases 22 25) with extremely low HbO_2 values were moribund at the admission and the clinical condition was not improved by artificial ventilation One patient case 29) was panicstricken when lying in the respirator it was impossible to obtain effective ventilation and he died after 2 days Three patients (cases 16 21 28) improved initially but died after 4 11 days Complicating diseases presumably largely determined the fatal course in 2 of these patients (a large pulmonary embolus in case 21 and a bleeding duodenal ulcer in case 28) In one patient the treatment was severely complicated by abundant bronchial secretions (case 16) In 9 of the 12 patients surviving the acute stage, the average duration of the artificial ventilation until stabilization of the venous plasma CO_2 content was 14 days the average CO_2 content being 32 mEq/l In the remaining 3 patients (cases 15, 17, 23) 2 of them being less intensively ventilated (cases 17 23), the CO_2 content remained high for a prolonged period or did not fall at all

Generally the arterial PCO_2 values were higher, and the pH and HbO_2 values lower in the patients with chronic bronchitis (table II) than in those with pulmonary tuberculosis (table I). In the first group 5 showed HbO_2 values below 40 per cent, in the latter none. Two patients with HbO_2 values below 30 per cent (cases 22, 25) (table II) died within a few hours after the admission, possibly due to irreversible hypoxic lesions (13).

The arterial blood samples were usually taken several hours, in some cases a few days before the admission to the Department of Epidemiology. In the meantime a further deterioration of the clinical condition had usually taken place. There is therefore no definite relationship between the blood-gas values and the state of consciousness at the start of artificial ventilation. On the whole pH is only moderately reduced and the plasma CO_2 content is high. This shows that the respiratory acidosis is fairly well compensated, and indicates that the pulmonary failure has generally not developed in a very acute manner.

No complications such as gastric dilatation or circulatory failure due to the treatment were observed. Seven semiconscious patients showed severe restlessness and needed sedation.

Most of these patients suffered from far advanced chronic lung disease. After the treatment described above, they have shown a tendency towards recurrent pulmonary failure, and many of them have therefore been treated with artificial ventilation several times. However, in view of the regular clinical control in these cases, the artificial ventilation has usually been instituted when the degree of pulmonary failure was less severe.

Three patients, all with arrested tuberculosis, who have been dependent on prolonged hospitalization and artificial ventilation several times every year for 11, 7 and 2 years, respectively, have tank respirators at home (cases 1, 2, 8). They sleep in the respirators every night, and during day they are working full time in light jobs.

Of the 26 patients surviving the acute stage 13 are still alive after an average observation time of 3 years.

Among the 14 patients with pulmonary tuberculosis and thoracoplasty (table I), 7 are alive after an average observation time of 4 years, while 7 died after an average observation time of 2 1/2 years, 3 of them, with severely reduced pulmonary function, died from cardio pulmonary failure unrelieved by continuous ventilation in tank respirator (cases 3, 5, 10), 1 from pulmonary failure in another hospital (case 6).

Of the 12 patients with chronic bronchitis, 6 are still alive after an average observation time of 2 years, while 6 died after an average observation time of 1 year.

Discussion

In the present series, including 32 patients with severe pulmonary failure, 27 patients were unconscious or semiconscious before the start of artificial ventilation in tank respirators.

Six of them died in the course of the first 3 weeks but in 2 of them factors other than the pulmonary failure *per se* probably largely determined the fatal

course The series represents a selection of severely diseased patients, being admitted for artificial ventilation due to failure of other therapy It seems probable that a high percentage of the patients would have died within a short time if artificial ventilation had not been instituted

It is difficult to compare the present series with others In order not to precipitate carbon dioxide narcosis only few of the patients received oxygen before the start of artificial ventilation The observed hypercapnia was therefore less pronounced than in other series, where the hypoxemia had been relieved and carbon dioxide narcosis developed (3, 7) On the other side, the hypoxemia was generally of severe degree, many patients showing oxygen values at the lower limit compatible with prolonged survival (12) Generally the clinical condition deteriorated further during the time elapsing between the blood gas examinations and the start of artificial ventilation With respect to acute mortality these results correspond to the results obtained by others with tracheotomy and intermittent positive pressure breathing (3 4 7)

Both for acute and for long term survival the results obtained were better in the patients with pulmonary tuberculosis and predominantly restrictive pulmonary insufficiency than in those with chronic bronchitis and predominantly obstructive insufficiency This in respect of acute mortality may possibly be due to the pulmonary failure being more severe in the latter group (table II) as judged by the severity of the disturbances of consciousness and by blood gas values It may also have played a role that the chronic bronchitis group comprised older patients, and that the pulmonary failure in these patients to a higher extent represented the final result of a gradual decrease in cardio pulmonary function, while in the chronic tuberculosis group (table I) the acute failure in the majority of patients was provoked or intensified by factors such as pneumonia or treatment with oxygen or analgesics which depressed the ventilation However, it cannot be excluded that the artificial ventilation, as routinely performed with relatively moderate negative pressures (*vide infra*) was less adequate in the patients with chronic bronchitis

A CO_2 content of 30 mEq/l or below was achieved in 1/4 of the patients surviving the acute stage In 1/3 of the patients however, the venous plasma CO_2 content remained above 34 mEq/l, even after weeks of artificial ventilation In some patients this was probably due to insufficient artificial ventilation, in others there may have been delayed normalization of the renal compensatory mechanisms, or processes of primary metabolic alkalosis may have been superimposed on the respiratory acidosis (10 11, 14)

As a standard method for artificial ventilation in patients with pulmonary failure due to chronic lung disease the tank respirator is preferred to positive pressure breathing for the following reasons

The method does not necessitate tracheotomy and is not dependent on an airtight fitting tracheal cannula, complications are avoided, such as superinfection of the airways, damage to bronchial epithelium by frequent suction

or inhalation of dry air tracheal stenosis and difficulties such as loss of ability to talk and cough The abundant bronchial secretions frequently seen in unconscious severely underventilated patients can be removed by suction through a tracheal tube when adequate ventilation is first established the secretions usually decrease and there is no further need for frequent suction

The tank respirators are very convenient for repeated artificial ventilation the treatment can whenever necessary be instituted by the nursing staff Patients with a strong tendency towards severe pulmonary failure may be ventilated during the night or parts of the day and have the rest of the time free for light activities this may go on for years even outside the hospital

Lack of success with artificial ventilation in tank respirators is in many cases probably due to application of too low pressures and ventilatory frequencies In other studies it has been observed that in patients with a minimum of cardiopulmonary function and stiff lungs it may be necessary to apply negative pressures up to about 40 cm H_2O and frequencies up to 28 per minute in order to obtain adequate ventilation (11) i e about three times the settings used in the treatment of patients with paralytic hypoventilation but normal lungs Many tank respirators are not designed for working at such high pressures and frequencies and may therefore be less suitable for treatment of this type of patient With frequent blood gas determinations and more individualized adjustment of the pressures and the frequency of the respirators the results can probably be better than in this series

Summary

Thirty two patients with severe hypercapnia and hypoxemia due to chronic lung disease have been treated with artificial ventilation in standard tank respirators Among 14 patients with chronic pulmonary tuberculosis and thoracoplasty 12 were unconscious or semiconscious at the start of artificial ventilation all survived the acute stage Among 18 patients with chronic bronchitis 15 were unconscious or semiconscious 6 died in the first 3 weeks 2 of them due to complicating diseases

Seven patients with pulmonary tuberculosis and 6 with chronic bronchitis are still living after average observation times of 4 and 2 years respectively while 7 with pulmonary tuberculosis and 6 with chronic bronchitis died after average observation times of 2 1/2 and 1 years respectively The advantages of using tank respirators in the treatment of pulmonary failure due to chronic lung diseases are emphasized

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Megaloblastic Anaemia Associated with Anticonvulsant Drugs

Studies on a Case with Four Relapses

By

ARNE HAMFELT, ANDREAS KILLANDER, ELIS MALERS and CARL HENRIC DE VERDIER

The first reports of megaloblastic anaemia during anticonvulsant drug therapy were published by Mannheimer and associates in 1952 and by Badenoch in 1954. Since then about 75 cases have been described. In spite of numerous studies performed on different patients, the mechanism remains obscure. The majority of patients have been investigated once, i.e. at the time of diagnosis. Only a few patients have been reported to develop megaloblastic anaemia twice while on anticonvulsant therapy.

In this paper we describe a patient in whom megaloblastic anaemia occurred four times during treatment with anticonvulsant drugs. These relapses provided unusual facilities for different investigations.

Case report

The patient, a man born in 1910, was completely normal until the age of 10, at which time a grand mal epilepsy started. Two twin sisters of his father had epilepsy since one year of age. Both parents were healthy. There were no siblings. The patient was first treated with phenobarbitone which he has received since then. In 1948 100 mg of diphenylhydantoin was added twice daily since the seizures were not well controlled by phenobarbitone alone. In 1950, when 40 years of age, he became ill with drowsiness, weakness and vomiting as dominant symptoms. His condition deteriorated in the course of a few months and he was sent to the County Hospital. On admission he was pale and weak. The temperature was 39.8 °C. Liver and spleen were not palpable. Blood investigation showed Hb 5.8 g %, red cells 1.65 mill./mm³, white cells 5 000/mm³ with a normal differential count. Bone marrow was not studied. The stools contained no blood on repeated examination. X-rays of the stomach were normal. No further laboratory investigations were performed. The patient was first treated with blood transfusions with only transient effect on the Hb concentration. Then iron was given without effect on the blood picture. Because of frequent badsmelling diarrhoea a sprue syndrome was suspected and on unchanged

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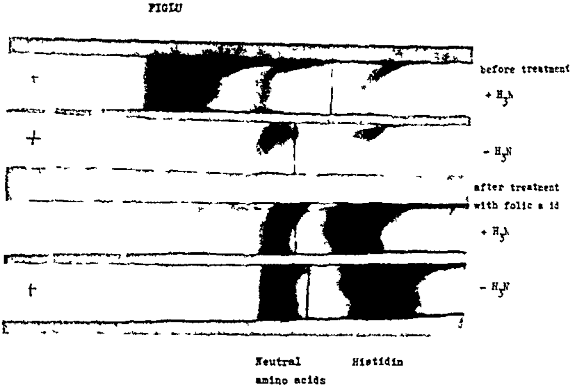


Fig 1 Paper electrophoresis of FIGLU in urine. The upper two strips show the appearance of a ninhydrine positive spot (glutamic acid) in the place of the FIGLU after reacting with ammonia (the upper strip) when the FIGLU is transferred to glutamic acid but no spot (FIGLU does not react with ninhydrine) when the reaction with ammonia has not been performed (the second strip). The two lower strips show the same investigation of the urine after treatment with folic acid *when no FIGLU could be found*

antiepileptic regimen folic acid treatment was started with 10 mg three times daily. The effect was remarkable. His general condition improved rapidly, the pyrexia and the diarrhoea disappeared and the Hb concentration and red cells increased to 12.2 g%, and 4.12 mill./mm³ respectively. No reticulocyte counts were performed. He was discharged on his usual *daily dose of phenobarbitone (100* mg × 2) and diphenylhydantoin (100 mg × 2). *No further folic acid treatment was* prescribed.

During the following eight years he felt fairly well but continued to have major seizures four to five times per month. In June 1958 the frequency of epileptic fits increased to three times a week and he began to feel weak. *The stools were loose and fre*quent. On July 1st he was admitted to the *University Hospital Uppsala*. Laboratory investigations showed Hb 9.8 g%, red cells 3.0 mill./mm³, white cells 8 500/mm³. In the blood smear there were a few nucleated red cells, basophilic stippling, anisocytosis and poikilocytosis. ESR was 22 mm/hour. Reticulocytes 3.1%. Platelets 102,000/mm³. The bone marrow was megaloblastic. The gastric juice contained free hydrochloric acid. The serum B_{12} concentration determined with *Euglena gracilis strain Z* (5, 7) was 195 (normal range 150—900 $\mu\mu$g/ml). X-ray *investigations of the digestive tract* were normal. Bilirubin, alkaline phosphatases and thymol in serum were within normal limits. Galactose tolerance test and liver biopsy were normal. The excretion of fat in faeces was normal. The red cell life span measured with radioactive chromium was also normal.

During unchanged antiepileptic therapy the patient was treated with oral folic acid 10 mg × 3 daily for seven days. The reticulocytes increased from 2.3% to 16.0%

on the sixth day. Three weeks after the beginning of the folic acid therapy the haemoglobin concentration was 15.8 g%. On September 18, 1958 the patient was sent home with a daily dose of diphenylhydantoin (Difhydan®) 100 mg × 3 and phenobarbitone 100 mg × 1, but no folic acid.

In July 1959 the phenobarbitone was increased to 150 mg daily and in Feb. 1960 to 200 mg daily. In July 1960 primidon (Mylepsin®) 250 mg × 3 was added to the previous medication *because of difficulties in suppression of the epileptic seizures*. Blood values were controlled in the Outpatient Department with intervals of about three months and found to be normal during 1959 and 1960. In Jan. 1961 the haemoglobin had decreased to 8.6 g% and the red cells to 2.6 mill./mm³. He had been tired, weak and drowsy the last two months. He had lost his appetite, had been nauseated and even vomited and he was therefore readmitted to the hospital. Physical examination showed a pale, slow-cerebrating man in no acute distress. Blood examination showed Hb 6.4 g%, red cells 2.1 mill./mm³, white cells 2,900/mm³. Differential count showed anisocytosis and poikilocytosis as well as moderate toxic granulation of the white cells. Platelets were 165,000/mm³. Sternal puncture revealed a megaloblastic bone marrow. ESR was 50 mm/hour. Liver function tests were normal, serum iron was 161 μg/100 ml and serum B_{12} concentration was 175 μμg/ml. The gastric j ce at th s t me onta ned no free hydrochloric acid after s mulat on w th 0.8 mg of histamine s bc aneous. Radioactive vitamin B_{12} absorption test according to Schilling (10) showed a urinary excretion of 18.3% of the oral dose (1.0 μg) in 48 hours. The folic a [illegible] n serum was determined n cr t f [illegible] all w I Lactobacillus casei l and was found [illegible] be 3.1 ng/ml. The normal values in this laboratory range from 3 to 1 [illegible] ng/ml serum. After a histidine load ac [illegible] ing [illegible] Knowles et al. (9) a m ch in creased ex [illegible] e on f f rm n noglutamic acid [illegible] n [illegible] ne was f [illegible] n as shown in fig. 1. Absorpt [illegible] n of [illegible] glucose and xylose was [illegible] pl tely normal.

Two weeks were required for these studies. During that time the patient's general condition gradually deteriorated. He had several periods of fever for which no obvious reason was detected. Hb and red cells decreased rapidly to 4.5 g% and 1.3 mill./mm³ respectively. The systolic blood pressure fell to 80 mm Hg and it became necessary to infuse 800 ml of whole blood.

In connection with the Schilling test the patient received 1 mg of vitamin B with no haematological response. He was then treated with folic acid in a daily dosage of 0.5 mg intramuscularly for four days. After this therapy his general condition improved strikingly, the number of reticulocytes increased to a peak of 15.8% on the eighth day and the haemoglobin and red cells rose to 14.3 g% and 4.5 mill./mm³. The histidine loading test showed no excretion of formiminoglutamic acid, as shown in fig. 1. A folic acid absorption test was unsuccessful because of technical difficulties. In connection with the test the patient received further 15 mg of folic acid parenterally for four days. The antiepileptic therapy was never interrupted during his stay at the hospital. On March 30, 1961 the patient was sent home with diphenylhydantoin 100 mg × 3, primidon 250 mg three times daily and phenobarbitone 200 mg at night. No folic acid was prescribed.

From March 1961 to Jan. 1964 the patient was followed regularly in the Outpatient clinic. He had nightly seizures three to twelve times per month and the medication was therefore changed several *times*. He ceased to take mylepsin in Sept. 1962 because of unsteadiness of gait. *In* March 1963 the dose was changed *to* 0.2 + 0.1 + 0.2 *g of di*phenylhydantoin per day and 0.2 g of *pheno*barbitone in *the evening*. Since then the frequency of epilep c f*ts decreased to* less than one per month. *The haemoglobin concentration varied* between 14.3 and 15.8 g% *from* March 1961 to March 1963. In Aug. 1963 *it* was 13.8 g%. However five months later on Jan. 21, 1964 a decrease of Hb to 10.6 g% led to a readmission of the patient for further investigation and treatment. On

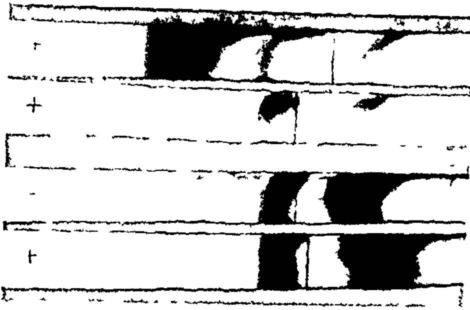


Fig 1 Paper electrophoresis of FIGLU in urine The upper two strips show the appearance of a ninhydrine positive spot (glutamic acid) in the place of the FIGLU after reacting with ammonia (the upper strip), when the FIGLU is transferred to glutamic acid but no spot (FIGLU does not react with ninhydrine) when the reaction with ammonia has not been performed (the second strip) The two lower strips show the same investigation of the urine after treatment with folic acid when no FIGLU could be found

antiepileptic regimen folic acid treatment was started with 10 mg three times daily The effect was remarkable His general condition improved rapidly the pyrexia and the diarrhoea disappeared and the Hb concentration and red cells increased to 12 2 g% and 4 12 mill /mm³ respectively No reticulocyte counts were performed He was discharged on his usual daily dose of phenobarbitone (100 mg × 2) and diphenylhydantoin (100 mg × 2) No further folic acid treatment was prescribed

During the following eight years he felt fairly well but continued to have major seizures four to five times per month In June 1958 the frequency of epileptic fits increased to three times a week and he began to feel weak The stools were loose and frequent On July 1st he was admitted to the University Hospital Uppsala Laboratory investigations showed Hb 9 8 g% red cells 3 0 mill /mm³ white cells 8,500/mm³ In the blood smear there were a few nucleated red cells basophilic stippling anisocytosis and poikilocytosis LSR was 22 mm/hour Reticulocytes 3 1 % Platelets 102,000/mm³ The bone marrow was megaloblastic The gastric juice contained free hydrochloric acid The serum B_{12} concentration determined with Euglena gracilis strain Z (5 7) was 195 (normal range 150—900 μμg/ml) X ray investigations of the digestive tract were normal Bilirubin, alkaline phosphatases and thymol in serum were within normal limits Galactose tolerance test and liver biopsy were normal The excretion of fat in faeces was normal The red cell life span measured with radioactive chromium was also normal

During unchanged antiepileptic therapy the patient was treated with oral folic acid 10 mg × 3 daily for seven days The reticulocytes increased from 2 3 % to 16 0 %

TABLE I

Meg anaemia	Interval	Year	Treatment	Daily dose (g)
		1920	Phenobarbitone	0·1—0·3
	28 years			
		1948		
	2 years			
Onset		1950		
			Phenobarbitone +	0·2
			Diphenylhydantoin	0·1—0·2
	8 years			
Relapse		July 1958		
	2·5 years	July 1960		
			Phenobarbitone +	0·2
Relapse		Jan 1961	Diphenylhydantoin +	0·3
			Primidone	0·75
	3 years	Sept 1962		
			Phenobarbitone +	0·2
			Diphenylhydantoin	0·5
Relapse		Jan 1964		

decreased and later on nausea and even vomiting occurred which might have interfered seriously with his food intake. However, the present evidence points to this situation being the result rather than the cause of the progressive anaemia. Repeated examinations failed to show any signs of malnutrition or of general intestinal malabsorption. Nor was there any indication that the absorption of folic acid was impaired, at least not during the last relapse in 1964. The serum folic acid activity was low normal during the relapse in 1961 and very low in 1964. The excretion of FIGLU after a histidine loading test was markedly increased at both these occasions. Complete haematological remission was induced by treatment with folic acid at all the four relapses. In these respects our patient resembles those reported previously by different investigators (8). Thus there seems to be little doubt that the anticonvulsant therapy provoked the recurrent bouts of megaloblastic anaemia in this patient.

Some features may deserve special attention. The intervals between the relapses and the association with therapy can be studied by dividing the history of his disease into different periods (table I). From table I it is evident that there was no anaemia during the first 28 years of treatment. The onset occurred 2 years after the addition of diphenylhydantoin. While on this type of combined therapy

admission he was pale, tired and apathetic His mental state had obviously deteriorated in comparison with previous occasions This time it was almost impossible to obtain adequate answers and he was rather dis oriented Hb was 9 6 g%, red cells 2 4 mill / mm³, white cells 3,300/mm³ The blood smear showed anisocytosis and poikilocytosis, a few megaloblasts and hypersegmented neutrophils The platelets were 120,000/mm³, reticulocytes were 0 2 % The bone marrow was frankly megaloblastic The serum B_{12} level was normal, 215 μμg/ml but the serum folic acid activity very low, 0 6 ng/ml After ingestion of 15 g L histidine hydrochloride he excreted 98 mmoles of formiminoglutamic acid in the first eight hours Determination of pyridoxal phosphate concentration in plasma (3) was performed as well as in erythrocytes and leucocytes The plasma value was 1 4 ng/ml (normal range 5—15 ng/ml), the con centration in the erythrocytes was 0 6 ng per 10^{10} red cells (normal range 3 0—18) and in leucocytes 0 17 ng per 10^{6} (normal range 0 1—0 3 ng/10^{6} cells) The glutamic oxalic acetic acid transaminase activity (GOT) in the leucocytes was 98 10^{-9} μmoles/hour/cell and in the erythrocytes 1 6 10^{-9} Fourteen control subjects without blood disease had 131 10^{-9} (range 47—252) μmoles/hour/cell and 5 8 10^{-9} (range 2 2—14 3) respectively A tryptophane tolerance test was performed using a dose of 100 mg per kg body weight The patient received 7 8 g L tryptophane by mouth in a glass of fruit juice The urinary excretion of xanthurenic acid (11) was 33 mg per 24 hours (2 0 μmoles per kg body weight and 24 hours) which is a normal value (less than 5 0 μmoles)

These studies required about two weeks during which time the patient was given the regular hospital diet However, he vomited several times a day and it became impossible to control the antiepileptic medication There was no increase of reticulocytes Hb fell to 7 1 g% and the red cells to 1 8 mill /mm³ Following this control period of two weeks treatment was started with pyridoxin (Benadon®) 40 mg three times daily for 10 days There was no change in the blood values The reticulocyte count varied between 0 and 0 4 % Thereafter 10 μg of folic acid in 1 ml was injected intramuscularly once daily for 14 days The folic acid solution was prepared by diluting a commercial preparation (Folvite®) containing 15 mg ml 1 to 1500 in physiological saline The reticulocyte count began to rise on the 4th day and reached a peak concentration of 31 8 % on the 7th day of treatment After two weeks the Hb con centration had increased from 6 0 to 9 5 g% and the haematocrit from 21 to 33 % During the following two weeks a mistake in preparing the folic acid solution made the patient receive 45 μg of folic acid daily in stead of 10 μg as originally planned Four weeks after the beginning of the folic acid therapy an absorption test was performed according to Chanarin et al (2) One hour after administration the serum level rose from 33 to 260 ng/ml In successive hours the level fell until 5 1/2 hours after the initial dose it was 60 ng/ml The blood picture was now completely normal and his general condition much improved The patient was therefore discharged on his regular schedule of anti epileptic treatment with the addition of a daily dose of folic acid

Discussion

It is evident that folic acid cured the patient's megaloblastic anaemia Vitamin B_{12} had no effect when tried in 1961 Moreover the serum-levels of B_{12} were consistently normal as was also the Schilling test The patient therefore seems to have suffered from repeated attacks of folic acid deficiency This might have been caused by dietary in sufficiency He lived with his parents who looked after him carefully and although the regular food in the house contained little vegetables it can prob ably not be judged as grossly deficient with regard to folic acid In the begin ning of the relapses the patient's appetite

there is evidence that pure folic acid deficiency plays an important role in the development of the megaloblastic anaemia, as shown by repeated abnormal serum folate and FIGLU assays. These findings are further supported by the rapid haematological response following the administration of very small doses of folic acid, 0.5 mg and 10 µg daily during the third and fourth relapses respectively.

Summary

An epileptic patient with four relapses of megaloblastic anaemia during treatment with anticonvulsant drugs is described. The patient had low normal to markedly decreased values of folic acid in serum, pathological histidine load test and no signs of malabsorption. The anticonvulsant drug therapy is thought to be responsible for the repeated bouts of megaloblastic anaemia which was cured with a daily dose of only 10 µg folic acid intramuscularly.

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he relapsed on two further occasions, i e 1958 and 1964 The relapse in 1961 was preceeded by about 6 months therapy with primidone as well as the other two remedies Of the three drugs used phenobarbitone and diphenylhydantoin were consistently associated with megaloblastic anaemia On the other hand phenobarbitone alone was administered for a period of 28 years with no haematological complications It thus appears either that diphenylhydantoin provoked the anaemia alone or in combination with phenobarbitone

Megaloblastic anaemia occurred for the first time two years after institution of combined therapy At that time the patient was given a total amount of 240 mg of folic acid during eight days At the relapse 8 years later 210 mg of folic acid was administered during one week, and in 1961 he was given 65 mg during nine days The highest amount of folic acid given was followed by the longest free interval On the other hand the two last free intervals were about the same length in spite of rather great differences in amounts of folic acid given These facts do not allow any conclusions regarding a possible connection between the length of the free intervals and the amount of folic acid given

Although the association between anticonvulsant drug therapy and megaloblastic anaemia seems to be fairly well established the mode of action is obscure A folic acid antagonistic effect of these drugs has been suggested by several authors but no experimental evidence for such a mechanism has been presented (8) Studies on microbiological systems have yielded conflicting results A possible inhibitory effect of phenobarbitone, diphenylhydantoin and primidone as well as of daraprim® and a tri amino pteridin was studied on dihydrofolate reductase, tetrahydrofolate formylase and methylenetetrahydrofolate dehydrogenase extracted from pigeon liver (4) The anticonvulsant drugs showed no inhibition in contrast to the other two substances which inhibited dihydrofolate reductase These drugs, also known to cause megaloblastic anaemia in a small number of patients, had an inhibitory effect which was calculated to be about 500 times weaker than that of aminopterin

The most important pathway for incorporation of one carbon unit into the one carbon pool passes via serinealdolase which has tetrahydrofolic acid and pyridoxal phosphate as cofactors 5 metyltetrahydrofolic acid is formed from the reaction product and stored mainly in the liver (6) Deficiency of pyridoxal phosphate or inhibition of enzymes using pyridoxal phosphate as a co factor would thus interfere with storage of folic acid The possibility of such a mechanism was explored by determination of pyridoxal phosphate in the patient s erythrocytes and leukocytes Low values were found in plasma and erythrocytes in which low values of GOT were found too A subsequent tryptophane tolerance test was normal Thus there was no clear evidence of impaired vitamin B_6 function Furthermore a therapeutic trial with pyridoxine had no effect on the blood picture

These studies do not prove nor do they exclude the presence of the suggested anti folic acid effect of antiepileptic drugs However in this case

there is evidence that pure folic acid deficiency plays an important role in the development of the megaloblastic anaemia, as shown by repeated abnormal serum folate and FIGLU assays These findings are further supported by the rapid haematological response following the administration of very mall doses of folic acid 0 5 mg and 10 µg daily during the third and fourth relapses respectively

Summary

An epileptic patient with four relapses of megaloblastic anaemia during treatment with anticonvulsant drugs is described The patient had low normal to markedly decreased values of folic acid in serum pathological histidine load test and no signs of malabsorption The anticonvulsant drug therapy is thought to be responsible for the repeated bouts of megaloblastic anaemia, which was cured with a daily dose of only 10 µg folic acid intramuscularly

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Adams-Stokes Syndrome in Children Without Organic Heart Disease

Electrocardiogram after Exercise as a Diagnostic Tool

By

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Spells of unconsciousness are fairly common symptoms in pediatric patients Most often the attacks turn out to be of epileptic origin but in every instance a differential diagnosis has to be made from several other conditions such as hypoglycaemia hyperventilation syndrome orthostatic hypotension and Adams Stokes syndrome

This paper will consider the diagnosis of Adams Stokes syndrome in children and especially in children without organic heart disease The inspiration to the paper was given us by the case history of a 7 year-old girl who presented with repeated syncopal attacks during exertional strain At the outset no sign of cardiac disease was revealed and as the electroencephalogram was slightly abnormal a diagnosis of epilepsy was proposed However later on the diagnosis was corrected to Adams Stokes syndrome because of the result of an electrocardiogram obtained after heavy exertional strain

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Case report

Girl born 1956 was admitted on Jan 4th 1964 for evaluation of episodes of unconsciousness

There was no history of congenital heart disease or epilepsy in the family She was the second of three children the other two siblings being alive and well The pregnancy and delivery were normal The birth weight was 3 600 g Growth and development were normal and apart from the common childhood diseases she had been well until May 1962

From May 1962 until Dec 1963 she had 6 attacks of sudden loss of consciousness all in connection with straining — fast running fighting etc — during which she fell unconscious to the ground without warning being pale slightly cyanotic around the mouth totally flaccid without respiration She recovered after 3—5 minutes sometimes first after having received mouth-to-mouth artificial respiration She had incontinence of urine every time she bit her tongue once as she fell on her face and a few times she received excoriations of the skin She had total amnesia for the attacks After the attacks she slept for several hours and was tired the rest of the day There were no symptoms or complaints in the intervals

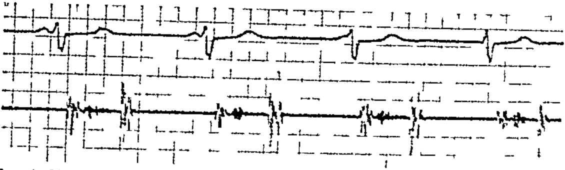


Fig 1 Phonocardiogram from 2nd left intercostal space showing mid systolic short ejection murmur and normal second heart sound ECG (lead I) shows wandering pacemaker

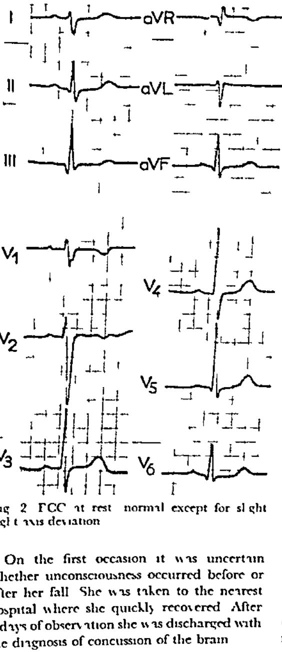


Fig 2 ECG at rest normal except for slight right axis deviation

On the first occasion it was uncertain whether unconsciousness occurred before or after her fall She was taken to the nearest hospital where she quickly recovered After 3 days of observation she was discharged with the diagnosis of concussion of the brain

The second attack occurred half a year later in Jan 1963 She was then referred by her doctor to a neurological out patient department and was examined on Feb 11, 1963 Physical examination revealed no neurological abnormalities and a roentgenogram of the skull was normal EEG was slightly abnormal (unprovoked too much activity of low frequency, during photic stimulation paroxystic 3—4 hz activity with small spikes especially in the occipito-parietal regions) The attacks were thought to be epileptic seizures and she was treated with phenobarbital 50 mg every evening

In spite of the anti-epileptic treatment she had her third attack in June 1963 her fourth and fifth in Aug and her sixth in Dec 1963

She was referred to the University Clinic of Pediatrics on Jan 4 1964 Physical examination showed no signs of neurological disease The EEG was essentially identical to the one obtained in Feb 1963 The concentration of phenobarbital in her serum was found to be 5 μg ml showing that the anti epileptic medication had been given at home Heart auscultation gave only a faint low pitched murmur in the 2nd and 3rd left intercostal space A phonocardiogram from the pulmonary area is shown in fig 1 It appears that the splitting of the second sound varied normally during respiration and that the murmur had all the characteristics of an innocent pulmonary flow murmur The chest roentgenogram showed normal conditions Electrocardiogram at rest (fig 2) showed slight right axis deviation and transiently wandering pacemaker (cf fig 1) but otherwise no abnormality was revealed According to

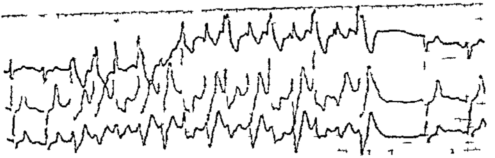


Fig. 3. Leads V[illegible], V[illegible] and V[illegible] of ECG immediately after heavy exercise: runs of multifocal ventricular extrasystoles with a few normal beats at the beginning and at the end.

the referred findings it was supposed that the child had no cardiac disease, but as her attacks were intimately linked with exertional strain it was felt warranted to obtain an ECG after exercise. First the patient exercised on a bicycle ergometer for 2 minutes with a load of 300 kg m/min. She was not fatigued by the work and the ECG obtained after the exercise was normal. A new work test was performed. This time she performed a rather heavy work (300 kg m/min for 2 minutes followed by 500 kg m/min for 5 minutes). She was very fatigued by the work, and an ECG obtained immediately after the exercise showed runs of 12–14 ventricular extrasystoles of multifocal origin interrupted by a few normal beats (fig. 3). After about one minute the rhythm returned to normal. No signs of cerebral ischaemia were observed.

Now the diagnosis was corrected from epileptic seizures to Adams-Stokes syndrome. The antiepileptic treatment was stopped and she was told to avoid exertional strain. She was discharged and one month later she had a new attack which as the previous was released by heavy exertional strain. On April [illegible] 1984 she was readmitted for a follow-up examination. The ECG now showed absolutely normal conditions, while an ECG after heavy exercise still revealed runs of multifocal ventricular extrasystoles for about one minute.

Discussion

Adams-Stokes syndrome is now generally defined as attacks of acute cerebral ischaemia due to an inadequate circulation caused by a sudden change in cardiac rhythm (8). The cerebral ischaemia may lead to unconsciousness with or without convulsions and long lasting attacks may lead to irreversible cerebral damage or death. Cardiac arrhythmias of highly different character may provoke the syndrome (8): prolonged runs of extrasystoles, different tachycardias, sometimes precipitated by extrasystoles such as ventricular tachycardia, ventricular flutter or a combination, bradycardia and ventricular asystole, sometimes following tachycardia.

The syndrome has been reported several times in children with congenital atrioventricular block (5, 12, 14, 16, 20) and in children with atrioventricular block due to diphtheria, rheumatic fever and different infectious diseases (3, 4, 6, 10, 11, 15, 17, 18) as well as in acute haemorrhage (13). Reports concerning Adams-Stokes syndrome in children

without organic heart disease are rare (1, 2, 7, 9, 19)

Berg (2) presents the histories of three sisters suffering from frequent attacks of multifocal ventricular extrasystoles without other signs of organic heart disease, two of the patients had Adams Stokes attacks from the age of 14 and 8 years, respectively, and the latter died suddenly while bicycling Frequent ventricular extrasystoles were present at rest, and the attacks were not related to exercise In the former patient the attacks were controlled with quinidine and procainamid Berg ascribes the arrhythmia to some unknown congenital disorder of the myocardial metabolism

Akaishi and Yokoyama (1) report the histories of two children with episodic attacks of unconsciousness diagnosed as Adams Stokes syndrome The first patient was a 9 year-old boy with syncopal attacks for 4 years caused by exercise and emotional stress At rest the electrocardiogram showed transient atrioventricular dissociation, during an exercise test multifocal ventricular extrasystoles appeared and the boy lost consciousness The electrocardiogram then successively showed ventricular fibrillation, atrioventricular block, sino atrial block, asystole for 30 seconds, then sino atrial block and supraventricular tachycardia composed of mixing forms of right and left bundle branch block After 5 minutes the patient regained consciousness while the electrocardiogram showed ventricular extrasystoles in bigimeny, decreasing in frequency with return to normal rhythm after 17 minutes The patient's sister had similar syncopal attacks and died when 10 years old The second patient was a 10 year old girl with complaints of tachycardia and syncopes for 8 months On the day of admission she had an attack while an electrocardiogram was obtained, it showed atrial flutter with 2 1 block, then 1 1 conduction when she lost consciousness for 1 minute Quinidine was given and the arrhythmia was gradually relieved In the course of reconvalescence the electrocardiogram showed atrial flutter, Wenckebach phenomenon after 6 days and normal rhythm after 9 days

On admission the chest roentgenogram revealed cardiac enlargement, but after 2 weeks it returned to normal In neither of the patients were other signs of heart disease found

Horan and Venables (7) present the history of a 6-year-old girl first seen in 1959 with fainting spells for some 18 months The attacks were related to fear and exercise and were treated in vain as epilepsy for nearly one year An attack was finally observed in the hospital, and an electrocardiogram established the diagnosis of Adams Stokes syndrome The arrhythmia which caused the attacks varied in pattern from supraventricular tachycardia with variable complexes to alternating bi-directional complexes of uncertain origin and ventricular fibrillation Between the attacks no cardiac abnormality was found After several medications including quinidine had failed to control the attacks, the patient was finally treated with success by a herbalist with medication of unknown nature During a follow-up examination in 1961 the electrocardiogram still showed abnormal beats and tachycardia after exercise, but no attack was evoked

Johansson (9) investigated an 11-year-old boy with syncopes for some years related to fatigue and emotional stress. The electroencephalogram was normal. His pulse was palpated during an attack and it turned out that he at the beginning of the attack had a very slow pulse rate changing to a tachycardia. At rest the electrocardiogram showed sinus bradycardia, but after an exercise test an abnormal tachycardia was revealed. Quinidine had no effect but the attacks were completely controlled with a long acting isoproterenol medication.

Ward (19) reports about 2 siblings with attacks of unconsciousness precipitated by exertion. At rest the only cardiac abnormality was a prolonged Q T interval in the electrocardiogram. The elder one a 6-year-old girl with attacks since the age of 16 months was first thought to have epilepsy or breath holding attacks, and later on a psychological basis was suspected finally an exercise test was performed during which she lost consciousness while the electrocardiogram showed abnormal complexes leading to ventricular fibrillation. After some trial treatments the attacks were controlled by an inhibitor of the beta receptors of the sympathetic system pronethalol (Alderlin). Her 17 month-old brother then began to have similar attacks which were harder to control. A newer beta receptor inhibitor was tried but as it made the child irritable the parents withheld the drug and 18 hours later he had a fatal attack. Another brother aged 6 years and the father were healthy with normal electrocardiogram but the mother showed a prolonged Q T interval and the mother's sister had had attacks of loss of consciousness during one of which she died at the age of 30.

As to our patient, who at rest presented no definite signs of heart disease, we are convinced that she, too, had Adams-Stokes syndrome. Her cerebral attacks were observed only during exertional strain, and repeated investigations demonstrated that heavy exercise led to runs of multifocal ventricular extrasystoles. Furthermore although the electroencephalograms first obtained were slightly abnormal, the last one was entirely normal after 3 months without anti epileptic treatment. The only doubt as to the diagnosis is that syncope was not observed during the exercise test. However, as it was possible to order the girl to perform a rather heavy work on a bicycle ergometer leading to short runs of ventricular extrasystoles it seems reasonable to presume that vivid amusing play with her friends might have stimulated her to still greater exertional achievements resulting in longer runs of extrasystoles or other tachyarrhythmias leading to diminished cardiac output and syncope. The slight abnormalities in the first electroencephalograms might have been due to cerebral damage caused by cerebral ischaemia or a head injury during one of the Adams Stokes attacks. The later normalisation of the electroencephalogram favours this hypothesis.

So far our patient has received no medical treatment for her tendency to tachyarrhythmia. We have ordered her several times to restrain her physical activity and perhaps she has now followed the advice.

Conclusion

The possibility of Adams Stokes syndrome in children should be kept in mind in dealing with spells of unconsciousness with or without convulsions, even if the physical examination, the resting electrocardiogram and the chest roentgenogram show no signs of heart disease The best way to diagnose Adams Stokes syndrome is to obtain an electrocardiogram during an attack or, as second best, to palpate the pulse at the onset of the attack If the syncope is related to exertional strain, the likelihood of a cardiac origin is especially high, and in such cases the tracing of an electrocardiogram during or after maximal exertional strain might be a highly valuable diagnostic tool as demonstrated in our patient

Summary

A survey is given of Adams Stokes syndrome in children without organic heart disease, comprising 8 cases from the literature and one personally investigated

Our patient was a 7-year old girl, who presented with repeated syncopal attacks during exertional strain Physical examination, resting electrocardiogram, and chest roentgenogram showed no sign of heart disease A misleading diagnosis of epilepsy was first made as the electroencephalogram was slightly abnormal

However, electrocardiograms obtained after heavy exercise showed runs of multifocal ventricular extrasystoles, supporting the diagnosis of Adams Stokes syndrome

The value of electrocardiography during or after heavy exercise is stressed

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Conclusion

The possibility of Adams Stokes syndrome in children should be kept in mind in dealing with spells of unconsciousness with or without convulsions, even if the physical examination, the resting electrocardiogram and the chest roentgenogram show no signs of heart disease The best way to diagnose Adams-Stokes syndrome is to obtain an electrocardiogram during an attack or, as second best, to palpate the *pulse* at the onset of the attack *If the syncope is related to exertional strain*, the likelihood of a cardiac origin is especially high, and in such cases the tracing of an electrocardiogram during or after maximal exertional strain might be a highly valuable diagnostic tool as demonstrated *in our patient*

Summary

A survey is given of Adams Stokes syndrome in children without organic heart disease, comprising *8* cases from the literature and one personally investigated

Our patient was a 7-year-old girl, who presented with repeated syncopal attacks during exertional strain Physical examination, resting electrocardiogram, and chest roentgenogram showed no sign of heart disease A misleading diagnosis of epilepsy was first made as the electroencephalogram was slightly *abnormal*

However, electrocardiograms obtained after heavy exercise showed runs of multifocal ventricular extrasystoles, supporting the diagnosis of Adams Stokes syndrome

The value of electrocardiography during or after heavy exercise is stressed

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From The Institute of Clinical Biochemistry and Medical Department B The University Hospital Rikshospitalet Oslo Norway

Hyperlipemia in Acute Adrenocortical Insufficiency

By

P Björnstad E Gjone and H N Haugen

To our knowledge hyperlipemia with lactescent serum in acute adrenocortical insufficiency has not been reported previously This paper deals with a patient suffering from Addison s disease in whom high levels of triglycerides and free fatty acids (FFA) were demonstrated during crisis Rapid clearing of the lactescent serum was observed after institution of steroid treatment

Case report

A 46-year-old tailor was admitted to Medical Department B Rikshospitalet Oslo on April 21 1964 He had always been of excellent health and had never been in hospital There was no known previous tuberculous illness His tuberculin reaction had been positive since childhood X ray examination of the chest performed yearly for the last 12 years had always been normal

For the last year he had noted an increased dark brown pigmentation of the skin During the last 3 weeks before admission he suffered from general asthenia muscle weakness and leg cramps increased fatigability anorexia nausea abdominal pain and breathlessness He lost 6 kg in weight in this period Two days before admission he started vomiting and was admitted to Oslo Military Hospital under the tentative diagnosis of acute abdomen A diagnosis of Addison s disease was made and he was immediately transferred to this department

On admission he looked very ill, weak and tired He vomited frequently The skin and tongue were dry There was increased pigmentation of the neck and elbows the sacral region and increased darkening of the palmar creases and of the gingival mucous membrane B P was 100/80 mm Hg the temperature normal The examination of the internal organs revealed no abnormal findings

The laboratory data were serum sodium 129 *mEq/l*, serum potassium 6 8 mEq/l, serum chlorides 96 *mEq/l* serum creatinine 2 3 mg/100 *ml* Hb 13 7 g/100 ml white blood cells 7600 *mm*³ ESR 20 mm/hour A calcified primary complex in the right lung was shown on X ray of the chest Intravenous pyelography was normal No adrenal calcifications could be seen on X ray examination Four urine cultures for tubercle bacilli were all negative

The history the findings of pigmentation and hypotension and the electrolyte disturbances were considered sufficient for the diagnosis of Addison s disease On account of his serious condition treatment was started im

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findings will be discussed in relation to the present knowledge of factors regulating plasma FFA and triglyceride concentration

Plasma FFA have their origin in adipose tissue and are removed mainly by the liver, heart and skeletal muscles The removal of FFA in these organs appears to be determined by the plasma concentration The level of plasma FFA is therefore largely determined by the release of fatty acids In adipose tissue there is a continuous lipolysis of triglycerides and a concomitant re esterification of free fatty acids to triglycerides and the release of fatty acids is determined by the balance between these two processes While lipolysis is known to be stimulated by a wide variety of hormones the re esterification process is largely determined by the insulin stimulated glucose utilization and formation of α glycerophosphate from glucose

Two of the most potent stimulators of lipolysis are adrenaline and nor adrenaline This catecholamine induced stimulation of lipolysis is however dependent upon cortisol and since our patient suffered from acute adrenocortical failure and consequently lack of cortisol a mechanism operating via the catecholamines is less probable

Adrenocortical insufficiency is always accompanied by increased secretion of ACTH It has recently been shown that ACTH exerts an extra adrenal stimulatory effect upon lipolysis and fatty acid release (3 11) Since our patient was critically ill a large secretion of ACTH is likely This may partly explain the elevated plasma FFA value Also TSH GH and three as yet unidentified pituitary substances (13, 19) have FFA releasing effects, but their role in adrenocortical insufficiency is unknown

It is known that plasma FFA increases during prolonged fasting, most likely as a result of decreased glycolysis resulting in a slowing down of the re esterification and a net increased lipolysis in adipose tissue Since our patient was in a state of prolonged fasting the increased level of plasma FFA may be due to inanition, a view which is supported by the fact that the FFA level remained unchanged until the patient could take regular meals

An increased level of FFA may also be explained by a decreased uptake in peripheral tissues In our patient the turn over time of FFA could not be studied because of the urgent need for specific therapy However no hormones with stimulatory or inhibitory effects on FFA utilization are at present known

The initial hypertriglyceridemia in our patient is compatible with an increase in the very low density, triglyceride rich lipoprotein fraction with Sf 20—400 It now seems established that plasma triglycerides in the fasting state are derived from the liver, and that the plasma FFA are the immediate precursors of the triglyceride fatty acids (6, 9 10)

The triglycerides formed in the liver are transported mainly as components of very low density lipoproteins — the Sf 20—400 fraction A large part of these triglycerides is removed at extravascular sites in peripheral tissues and the Sf 20—400 are converted to Sf 0—10 lipoproteins possibly by the action of the clearing factor lipase An increased level of triglycerides in serum as observed in our pa-

TABLE I. Plasma lipids before and during treatment of a patient suffering from acute adrenocortical insufficiency

	Triglycerides (mg/100 ml)	FFA (mEq/l)	Phospholipids (mg/100 ml)	Total cholesterol (mg/100 ml)
April				
21	322	1.02	259	238
22	180	1.07	235	249
23	212	0.83	216	210
27	168	0.55	221	228
May				
4	126	0.29	216	230

mediately by giving saline and hydrocortisone i.v. and cortisone acetate i.m. the first evening before hormone analyses could be done. Cortisone was thereupon given orally in smaller doses. The response to this treatment was rapid and excellent. Four days after admission the serum electrolytes were normal: sodium 141, potassium 3.9 and chlorides 102 mEq/l. The B.P. was 135/90 mm Hg. He was discharged after 15 days with a maintenance dose of 25 mg of cortisone acetate daily.

A very lactescent serum was obtained from the blood drawn on admission, but 12 hours after institution of treatment the serum was completely clear. Because of these findings, analyses of serum lipid fractions were performed several times during the patient's stay in the hospital. The results are presented in table I.

The triglycerides showed an increased level of 322 mg/100 ml, but fell rapidly during the first night to 180 mg/100 ml, whereas the decrease later was slower. Also the free fatty acids (FFA) were elevated: 1.02 mEq/l. No reduction was seen during the first night, but later the FFA showed a delayed decrease. A normal value of 0.29 mEq/l was obtained before the patient was discharged. The phospholipids and total cholesterol values were completely unchanged during the patient's stay in hospital.

The following analytical methods were used: Lipid phosphorus was estimated according to the method of Fiske and Subbarow (5) and the value multiplied by 25 to give phospholipids in mg/100 ml of serum. Total cholesterol was determined with the method of Carr and Drekter (1) and triglycerides according to Van Handel and Zilversmit (8). For the determination of free fatty acids the method of Dole (2) as modified by Trout et al. (17) was used.

Discussion

The effects of adrenocortical hormones on fat metabolism have been studied extensively in animal experiments. Observations of plasma lipid alterations in humans suffering from acute adrenocortical failure are, however, scarce.

During the last year we have observed lactescent serum in two patients suffering from acute adrenocortical failure with rapid clearing after treatment. This finding prompted a study of the plasma lipid fractions in the present patient both before institution of therapy and several times during his stay in hospital. The

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tient, may thus be due either to an increased production of very low density lipoproteins in the liver or to a decreased uptake of triglycerides from these lipoproteins in peripheral tissues The transport of triglycerides from the liver appears to be determined by the balance between uptake of FFA and liponeogenesis in liver on the one hand and the rate of FFA oxidation on the other It has been shown (12, 16) that elevated plasma FFA levels produce a consistent elevation of serum lipoproteins in experimental animals and in liver perfusion experiments (7, 14) However, increased FFA mobilization from depot lipids leading to hypertriglyceridemia also brings about a secondary elevation of plasma cholesterol and phospholipids Our patient had normal and stabile levels of these lipid fractions Moreover, the plasma triglyceride concentration fell rapidly and marked during treatment within the first 12 hours without change in the FFA level These findings, therefore, make it unlikely that the hypertriglyceridemia is secondary to an increased FFA mobilization Other explanations must therefore be considered Although contradictory results have been obtained by studying the effects of adrenal hormones on liponeogenesis and FFA oxidation in the liver (15, 18), recent experiments by Fain and Wilhelmi (4) have shown that adrenalectomy in rats did not result in any marked alteration of fatty acid synthesis in the liver It thus seems unlikely that these factors could contribute significantly to the observed hypertriglyceridemia in our patient

The most reasonable explanation of the observed hypertriglyceridemia in our patient seems to be a delayed uptake of triglycerides in the peripheral tissues Further discussion of this theory is at present not possible, since the effect of adrenal cortical hormones on the clearance of triglycerides and on the function of the clearing factor lipase is still unknown

Summary

A case of acute adrenocortical insufficiency with hyperlipemia and lactescent serum is reported High levels of plasma triglycerides and free fatty acids were present on admission During treatment with cortisol a very rapid fall in the triglyceride level and a more gradual fall in the FFA concentration were observed Cholesterol and phospholipid fractions remained within normal ranges Possible mechanisms to explain these alterations are discussed The patient's prolonged fasting and the FFA mobilizing effects of various hormones are thought to be the most likely explanations of the increased plasma FFA level, whereas a decreased uptake of neutral fat in peripheral tissues may explain the hypertriglyceridemia

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Myocardial Infarction During Anticoagulant Therapy

Relation of Anticoagulant Level to Presence or Absence of Recent Coronary Thrombosis at Autopsy

By

Kåre Molne and Peter F Hjort

Previously we have examined the efficacy of anticoagulant therapy in patients who died of acute myocardial infarction in spite of this therapy (10) Autopsy studies suggested that *recent coronary thrombosis* was more common in patients who were admitted to the hospital with evidence of insufficient anticoagulant therapy i e with high percentage levels in the PP test (16) or in the Thrombotest (15) However the material was too small for a statistical evaluation

Therefore we have reviewed a larger autopsy material of patients who died of acute myocardial infarction while they were on anticoagulant therapy In this material the PP or Thrombotest (TT) levels were significantly higher in those patients who had a recent coronary thrombus at autopsy than in those who did not have such a thrombus

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Material and methods

During the period from 1958 through 1963 a total of 11 320 autopsies were carried out in the Department of Pathology Ullevål Hospital All patients died in the Oslo City Hospitals By *routine techniques* the heart was opened with scissors along the septum following the blood flow The myocardium was sliced and inspected for necrotic and fibrotic lesions The coronary tree was then carefully opened with a pair of fine scissors and searched for stenoses and occlusions The findings were reviewed by a senior pathologist who collated myocardial and coronary lesions and classified thrombi in one of the following two groups

Recent thrombi are greyish red to bluish in color soft often clearly layered and loosely attached to the vessel wall

Old thrombi are reddish brown to light grey in color usually firm although sometimes soft and greasy fairly homogeneous and often well fixed to the vessel wall

In the period selected for study coronary disease was the main cause of death in 2 388 patients Of these 170 patients satisfied three criteria 1) they were on oral anticoagulant

patients the autopsy report described either a *recent* coronary thrombus or *no* recent coronary thrombus (i e either no thrombus or an old thrombus evidently not directly related to the final infarct)

The material is divided into two groups 52 patients (group A) had recent coronary thrombosis and 29 patients (group B) had no recent thrombosis Table I summarizes the pertinent clinical and pathological information

Sex In group A 23 % and in group B 24 % were women

Age The patients in group A were slightly older but not significantly so

Cholester l was estimated on or shortly after admission The level was elevated in both groups the difference between the groups was not significant

Hypertension had been diagnosed clinically with similar frequency in the two groups

Previous infarction Here are listed only those episodes which resulted in admission to hospital with confirmation of the diagnosis About 1/4 of the patients had no history of myocardial infarction and were on anti coagulant therapy because of angina pectoris or intermittent claudication More patients in group A (83 %) had a history of previous infarction than in group B (69 %) However at autopsy 73 % of the patients in group A and 72 % of the patients in group B had evidence of previous infarction viz a fibrotic area with the smallest diameter greater than 1 cm

Duration of the clinical history of arterial disease was sligthly longer in group B but the difference was not significant ($0.10 > p > 0.05$)

Duration of the anticoagulant therapy was similar in the two groups

Time from onset of the final infarction to death was also similar in the two groups

Heart weight was similar in the two groups

The mean anticoagulant level on admission for all patients was 29 6 % a value almost identical with mean level in our previous study 29 4 (10) However the mean level on admission differed significantly in the two groups 24 7 % in group B and 32 3 % in group A ($0.05 > p > 0.025$ by Student's t test) Thus patients who died of myocardial infarction with a recent coronary thrombus had a higher mean PP or TT level than patients who died of myocardial infarction without a recent coronary thrombus Table II and fig 1 give the distributions of the anticoagulant levels in the two groups

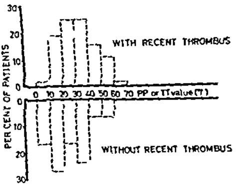


Fig 1 The percentage distribution of the PP or Thrombotest levels on admission The material is divided in two groups A 52 patients with recent coronary thrombosis B 29 patients without recent coronary thrombosis

Thirteen of the 29 patients in group B had a PP or TT level below 20 % while only 11 of 52 patients in group A were below this level This difference is significant ($0.05 > p > 0.025$ by χ^2 test) If the entire material is divided at the 15 % level nine of the 29 patients in group B had values below this level in contrast to only five of 52 patients in group A This difference is also significant ($0.05 > p > 0.025$ by χ^2 test) Thrombosis was therefore less common in adequately medicated patients

The distribution of age and sex was the same for patients with PP or TT levels above or below 20 % Further the sex proportion and the PP or TT- levels on admission were similar in patients above and below 60 years of age

Discussion

All patients in this material died of acute myocardial infarction in spite of anti

TABLE I Summary of clinical and pathological data on 81 patients

	A Recent thrombus	B No recent thrombus
No of patients	52	29
Sex	12 ♀, 40 ♂	7 ♀, 22 ♂
Age (yrs)	62.1 (39—82)	61.7 (49—73)
Cholesterol (mg %)	329 (194—520)	355 (170—683)
Hypertension (No of pat.)	8	6
No of previous infarctions		
1	36	19
2	7	1
Duration of history (months)	51.0 (2—180)	62.0 (4—192)
Duration of anticoagulant therapy (months)	30.2 (1—130)	31.3 (2—106)
Days between onset of infarction and death	7.3 (1—27)	8.8 (1—30)
Autopsy Old infarctions	38	21
Recent infarctions	52	29
Weight of the heart (g)	513 (360—770)	495 (300—780)
Mean PP or TT level (%)	32.3	24.7

TABLE II The distribution of the PP or Thrombotest levels on admission

	No of patients with a PP or TT level (%) of							Mean PP or TT level (%)
	<10	10—19	20—29	30—39	40—49	50—59	60—69	
A Recent thrombus	1	10	13	13	8	6	1	32.3
B No recent thrombus	5	8	5	7	2	2	0	24.7

therapy with phenylindanedione or with dicumarol when they had their final infarction, 2) the anticoagulant level had been determined by the PP test (16) or by the Thrombotest (15) when they were admitted to the hospital and 3) a recent myocardial infarction had been found at autopsy.

The clinical and autopsy records for these patients were reviewed and 89 patients had to be excluded for the following reasons. Fifty-five patients were excluded because the PP- or TT level obtained on admission may have differed from the anticoagulant level at the onset of the infarction. In 29 of these patients, more than 48 hours had elapsed between the probable onset of the infarction and the admission to hospital, in 26 patients, the onset of symptoms could not be determined. Nine patients were excluded because the infarction had been preceded by prolonged shock due to bleeding (5 patients) or other causes (4 patients). Fifteen patients were excluded because the pathologist had been unable to determine whether a coronary thrombus was recent or not. Finally, 10 patients were excluded because of a poor correlation between the location of the myocardial infarct and the coronary thrombus.

Thus 81 patients were left. They all died of acute myocardial infarction while they were on anticoagulant therapy and in all

Unfortunately, there is no evidence which rules out this explanation for our observation

Fourthly, the difference between the two groups *may* be due to a prevention of thrombosis by adequate anticoagulant therapy There is much evidence from animal experiments that anticoagulants do have this effect and we believe that this explanation is the most likely, even if other possibilities cannot be ruled out

What then, is the role of coronary thrombosis in the pathogenesis of myocardial infarction? The frequency of coronary thrombosis in patients who die of *myocardial infarction* varies markedly in the published autopsy materials 45 per cent (11), 53 per cent (3) 63 per cent (12) 69 per cent (8 23), 87 per cent (7), 92 per cent (18) The frequency of coronary thrombosis *in all cases of death from coronary disease* both with and without myocardial infarction, is usually lower 43 per cent (19), 47 per cent (14) 51 per cent (23) 52 per cent (5) These differences are probably due to method of selection autopsy technique and terminology In spite of these differences it is reasonable to conclude that coronary thrombosis plays a major role in at least 50 per cent of the patients who die of myocardial infarction It is conceivable that coronary thrombosis may at least in some patients develop *after* the infarction However even if this were so thrombosis would probably contribute to the development of the infarction Prevention of coronary thrombosis is therefore one of the main objectives of the therapy in coronary disease

Finally, what can anticoagulant therapy be expected to achieve? It cannot protect against acute non thrombotic mechanisms of death, and a significant proportion of patients with coronary disease die without a coronary thrombus Further, a thrombus differs from a clot and it takes less anticoagulant action to prevent coagulation than to block the formation of platelet thrombi (9 17) Therefore, anticoagulants can only be expected to offer a *relative* protection against thrombosis, and it is not surprising that some patients die of coronary thrombosis in spite of very low PP or TT levels However, controlled clinical trials have shown that anticoagulants do indeed offer *some* protection (2, 4, 20) We believe that the present study supports these clinical studies, since recent coronary thrombosis was less common in properly medicated patients The study also emphasizes, however, that patients on anticoagulant therapy should be maintained at the lowest PP or TT levels compatible with safety

Summary

From the autopsy records of Ullevål Hospital we have collected information on all patients who died of acute myocardial infarction from 1958 through 1963 For those who were on long term anticoagulant therapy we compared the autopsy and the clinical records in order to answer the question what is the relation between the anticoagulant level and the presence or absence of recent coronary thrombosis?

Eighty-one patients were suitable for analysis 52 had a recent coronary thrombus and 29 did not have a recent

coagulant therapy However, the incidence of coronary thrombosis was lower in adequately medicated patients Before conclusions can be drawn from this observation, four methodological problems must be considered

Firstly, are the pathological observations valid? The coronary arteries were examined by a routine technique which is not optimal Therefore, thrombi may have been lost or overlooked, and atheromatous abscesses or subintimal hemorrhages may have been misdiagnosed as thrombi We believe, however, that such errors tend to be equally distributed between the two groups

Secondly, is the material representative for patients on anticoagulant therapy? This question is pertinent, since so many patients were excluded However, these exclusions were all made according to pre-determined criteria The purpose of these criteria was to select a well-defined group of patients 1) who had died during anticoagulant therapy of an acute myocardial infarction which had been confirmed at autopsy, 2) who had been admitted to the hospital within 48 hours of the probable onset of infarction, 3) in whom a PP- or TT- level had been obtained on or shortly after admission, and 4) in whom the pathologist had concluded that there was either a recent coronary thrombus or no recent coronary thrombus Thus, the material is selected, but the selection is not biased

Thirdly, can the two groups be compared? Table I reveals several differences, but none are statistically significant, except the anticoagulant level on admission We believe, therefore, that the groups can be compared

Fourthly, do the PP- or TT levels on admission reflect the anticoagulant level during the infarction period? In a previous article (10) we showed that the anticoagulant level on admission was on an average 5 5 per cent higher than the last pre-infarction level This difference is so small that the level on admission can be taken to reflect the level which existed during the development of the infarction

We must conclude, therefore, that our observation *is* valid in patients who died of acute myocardial infarction during anticoagulant therapy a recent coronary thrombus was more common among those who had a high PP- or TT level on admission This observation is open to several possible explanations

Firstly, subintimal bleeding may conceivably be more common in patients who have an adequate or low PP- or TT-level than in those who have a high level Infarction in such patients could therefore be due to subintimal bleeding and not to thrombosis There was no evidence of this in our material, nor in the material of Fischer (6)

Secondly, anticoagulants accelerate recanalization of experimental thrombosis in animals (21, 22), and pre-mortal thrombolysis may therefore have removed the thrombi in patients with adequate anticoagulant therapy However, the survival time in most of our patients was probably too short for recanalization to occur

Thirdly animal experiments (1, 13) *suggest that insufficient anticoagulant* therapy may actually *increase* the tendency toward thrombosis over and above the tendency of untreated patients

Unfortunately, there is no evidence which rules out this explanation for our observation

Fourthly, the difference between the two groups may be due to a prevention of thrombosis by adequate anticoagulant therapy There is much evidence from animal experiments that anticoagulants do have this effect, and we believe that this explanation is the most likely, even if other possibilities cannot be ruled out

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From the autopsy records of Ulleval Hospital we have collected information on all patients who died of acute myocardial infarction from 1958 through 1963 For those who were on long term anticoagulant therapy we compared the autopsy and the clinical records in order to answer the question what is the relation between the anticoagulant level and the presence or absence of recent coronary thrombosis?

Eighty-one patients were suitable for analysis 52 had a *recent* coronary thrombus and 29 did not have a recent

coronary thrombus The first group had an average PP- or TT-level on admission of 32 3 %, the second group had an average level of 24 7 % This difference is statistically significant (0 05 > p > 0 025) We conclude from this observation that *adequate* anticoagulant therapy offers *some* protection against coronary thrombosis

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Diffuse Hodgkin's Disease of the Gastrointestinal Tract

Report of a Case with Profuse Steatorrhoea

By

E Scheffer and D Pott Hofstede

Attention was first drawn to the isolated gastrointestinal localization of Hodgkin's disease (lymphogranuloma malignum) by Schlagenhaufer in 1913 (38) In the following year a case of lymphogranuloma malignum limited to the duodenum the jejunum and the mesenteric lymph nodes was described by Eberstadt (11) Further publications about lymphogranuloma malignum limited to or mainly localized in the gastrointestinal tract and its regional lymph nodes have been reviewed by several authors (8 12 14 15 19 22 23 26 27 32 39) it is still considered to be a rare condition

We recently had an opportunity to observe a patient suffering from a profuse and incurable steatorrhoea resulting from a lymphogranuloma malignum localized diffusely throughout the gastrointestinal tract from the cardia of the stomach to the rectum and in the mesenteric lymph nodes No case with a similar diffuse localization has so far been encountered in the literature

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Case report

A 46 year old labourer (ditch digger) had enjoyed perfect health until the *spring of 1960* when he began to suffer from diarrhoea which occurred in periods alternating with periods of normal defaecation In a period of diarrhoea defaecation occurred 3 to 15 times a day thin grey stools being passed Blood or mucus were never seen The patient complained of abdominal pains of a colicky character and he was losing weight rapidly In *June 1960* the faeces were examined elsewhere and the diagnosis of steatorrhoea was made The patient was put on a gluten free diet which was only briefly beneficial At the end of *November 1960* he was sent as an outpatient to the Department of Internal Medicine of Leiden University Hospital By now he had lost some 22 kg the diarrhoea occurred 3 to 4 times a day he complained of tiredness felt sick and his mouth and tongue were painful X ray examination of the gastrointestinal tract revealed atonic intestinal loops with fluid levels The passage *time* was 6 hours There was flocculation The caecum and ascending *colon* were dilated A fat balance determination *was* unsuccessful because the patient did not keep to his diet On *December 8th 1960* the patient was admitted as an emergency case to the Department of Gastroenterology he ap-

peared sick, apathic, cachectic and severely dehydrated as a result of vomiting and increased diarrhoea

Physical examination of the abdomen revealed strong peristaltic movements No enlargement of the liver or of the spleen were felt, nor were any pathological lymph nodes found The patient had drum stick fingers His systolic tension was 80 mm Hg, his diastolic tension could not be measured X ray examination of the thorax revealed no changes The ECG presented signs of a low blood potassium level

Laboratory findings

Blood ESR 1/2 mm/h, Hb content 110 %, leukocytes 5,300, eosinophils 50, urea 28 mg %, sodium 122 mEq/l, potassium 4 mEq/l, chloride 93 mEq/l, HCO, 15 7 mEq/l, calcium 8 mg %, phosphorus 1 mg %, total protein content 3 4 mg/l, albumin 59 1 %, globulins α_1 9 5 %, α_2 8 8 %, β 7 3 %, γ 15 4 %, vitamin B_{12} 0 14 γ/ml, total bilirubin content 1 07 mg %, Hymans van den Bergh reaction indirectly positive, alkaline phosphatase 14 K A units, diastase 8 units blood sugar 77 mg %, haemostasis vitamin K deficiency

Urine 17 ketosteroids and 17 ketogens in 24 hour urine normal, 5 hydroxyindolacetic acid in 24 hour urine normal

Faeces Lugol and Sudan neg (no food intake) Benzidine reaction strongly positive, nitrogen excretion 5 to 6 g a day (without oral food intake), fat balance (Jan 1961) excretion 56 g in one day (food containing 49 g), no pathogenic micro organisms no amoebae no worm eggs

Fractional test on gastric juice no free acid protein present

Cytological examination of gastric contents no malignant cells

Finger print test (for increased excretion of sodium in the sweat) neg

The patient was treated with infusions, folic acid, vitamin B_{12}, A and D in order to normalize the electrolytes and to ensure a sufficient caloric supply He was given plasma and albumin as well he also received vitamin B-complex intramuscularly, magnesium sulphate intravenously, and calcium and salazopyrine per os He received nothing else per os, but was allowed to drink The patient soon became well hydrated but the tension remained low (± 90/70) The temperature, which had been normal, rose to 39° C Blood cultures were sterile, the Hb content now was 85 %, X rays of the thorax showed no infiltrative changes Cortisone (37 1/2 mg daily) was given for 6 days as at that stage adrenal insufficiency could not be ruled out

Thereafter ACTH was given instead, in a decreasing dosage from 80 I U to 20 I U daily, for 12 days followed by diadresone F intramuscularly (20—40 mg daily) and/or prednisone per os (20 mg daily)

The temperature returned to normal after 10 days The diarrhoea did not diminish 1 to 10 l of thin stools were passed daily and there was a great loss of electrolytes The blood level of the latter, however, could be regulated satisfactorily by means of infusions, and reasonable hydration could be maintained

On *December 15th, 1960* a severe intestinal haemorrhage occurred Probably the vitamin K-deficiency in combination with the heparin added to the infusion fluid (in order to prevent venesection thrombosis) played a contributory role here Treatment consisted of blood transfusion and vitamin K given intravenously

On *December 20th 1960* thrombosis appeared to have developed in the patient's right leg requiring anticoagulation therapy (Marcoumar)

Meanwhile the patient had slowly become icteric The total blood bilirubin content was 4 1 mg %, the Hymans van den Bergh reaction was directly positive The alkaline phosphatase was 26 I U (K A) Both these values decreased in the course of the illness but rose again shortly before death Transaminases were still normal, but in the later course the SGOT and the SGPT values increased

On *December 23rd, 1960* left sided pneumonia was diagnosed and treated with peni

cillin and streptomycin Haemophilus in fluenzae was isolated from the sputum

On *December 26th, 1960* the temperature rose and this time a staphylococcus aureus (coagulase positive) could be isolated from sputum and faeces The patient then received erythromycin and neomycin

The general condition did not improve the diarrhoea remained unaltered Since further observation and symptomatic therapy seemed senseless and since further diagnostic procedures (e g ileum biopsy) on this serious ly ill patient were impossible an exploratory laparotomy was decided upon in the hope of either finding a treatable alteration or obtain ing biopsy material for diagnostic purposes

On *January 2nd 1961* this laparotomy was performed Macroscopically no pathologic changes were found The intestines and the other abdominal viscera appeared normal Only some lymph nodes in the mesenteric root seemed slightly enlarged A few lymph nodes were excised Microscopically they showed the typical picture of Morbus Hodg kin (Dr R O van der Heul and Dr M J de Vries)

It was decided to administer mitoxin in travenously (total amount 21 mg)

On *January 10th 1961* the patient again had a high temperature and this time pneu monia was found on the right side he was treated with penicillin streptomycin and reverin A pneumococcus type V was iso lated from the sputum and the blood sputum cultures also yielded a staphylococcus aureus (coagulase positive) and a haemophilus in fluenzae The patient then received as well gammaglobulin given intramuscularly In this period the patient lost blood per anum Thrombocytopenia was found (40 000) dur ing a period of a few days He was given blood transfusions

On *January 15th 1961* there was no defaeca tion and the next day some almost formed stools were passed but then diarrhoea re appeared (2 to 7 l a day) and the patient steadily deteriorated

On *January 27th 1961* a new respiratory infection occurred this was treated with penicillin and reverin It was decided to ad minister T E M (5 mg twice a day) Thus, as could be expected proved unsuccessful within the short time available As the pa tient s condition was rapidly deteriorating he received X ray irradiation of the abdomen on five consecutive days (Feb 9th—14th) up to a total amount of 750 r

Eventually the icterus began to increase gradually and the patient became comatose on *March 15th 1961* he died the same day

Autopsy findings

Macroscopical findings The autopsy of this cachectic and icteric 46-year-old man (body weight 41 kg height 170 cm) revealed few if any macroscopical alterations suggestive of Hodgkin s disease There was no enlargement of any of the lymph nodes, even the mesenteric lymph nodes The liver and the spleen were not enlarged (weights 1,345 and 145 g re spectively), nor did they show any *specific* alterations when cut The liver presented diffuse fatty degeneration The gall bladder had a normal appearance The bile ducts were not distended nor were their walls infiltrated or compressed by any enlarged portal lymph nodes There were no bile concrements In the stomach the plicae were atrophic the mucosa was diffusely very atrophic (from cardia to pylorus) with many small islets showing less marked atrophy this gave the stomach a mottled appearance The in testines showed moderate dilatation and marked diffuse atrophy which continued from the stomach into the duodenum and involved the mucosa of the entire intestinal tract down to the rectum The appendix was thin (3—4 mm) *No tumours and no ulcerations* were found The oesophagus had a normal appearance the mucosa of the tongue was atrophic The thyroid was enlarged (weight 58 g) The thoracic and the other abdominal organs were quite normal except for the pleurae the visceral pleurae were adherent to the parietal ones The bone marrow did not show any changes Permission for skull dissection was not obtained

Microscopical findings The most conspicuous alterations were found in the *mucosa* of the *intestinal tract* Then it was realized that the



Fig 1 Gastric mucosa atrophy of the glands and of the lymphatic tissue

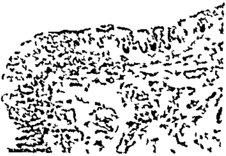


Fig 2 Mucosa of the small intestine there are no villi the lymphatic tissue also is atrophic

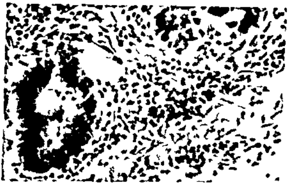


Fig 3 Infiltration of the jejunal mucosa atypical reticular cells some of which have hyperchromatic or pycnotic nuclei lymphocytes plasma cells eosinophils



Fig 4 Gastric mucosa with giant cell in the interstitial tissue between the glands This giant cell has characteristics of the Sternberg Reed cell two nuclei with coarsely dispersed chromatin and large nucleoli

same process had also afflicted the *stomach* (figs 1 and 2) The mucosa of the duodenum jejunum ileum caecum appendix and colon down to the rectum showed *extreme atrophy* in duodenum jejunum and ileum all traces of villi were lacking (fig 2) The crypts seemed to be greatly reduced in number and lay abnormally far apart The tissue between the crypts (fig 3) consisted of atypical reticular cells with scattered giant cells (some of them showing mitosis) many plasma cells lymphocytes neutrophils and eosinophils The nuclei of most of the giant cells appeared hyperchromatic and irregular in shape some giant cells showed an irregularly shaped vesicular nucleus (or more than one) with heavy lumps of chromatin and large often eosinophilic nucleoli thus presenting the characteristics of Sternberg Reed cells the cytoplasm was generally moderate in quantity but sometimes abundant and stained strongly with eosin

Many reticulum cells were atypical here and there real Hodgkin cells (28 31) (transition forms from reticulum cells to Sternberg Reed cells) were found (large vesicular nuclei with heavy nucleoli) This granulation tissue was found in all the sections made throughout the intestinal tract

A further striking observation was the advanced *atrophy of all lymphatic tissue* the lymph follicles over the entire gastrointestinal mucosa were meagre and scanty or in some sections absent Moreover atypical reticular cells were present in the lymphatic tissue The *oesophagus* appeared to be free of granulation tissue it showed only slight sub epithelial lymphocytic infiltration In

the *stomach* the microscopic alteration ex tended to the borderline between the gastric mucosa and the oesophageal epithelium This alteration was found in all the sections down to the pylorus We were struck in the first place by the marked atrophy of the entire mucosa (fig 1) patches of less at rophic mucosa alternating with areas of greater atrophy just as had been observed macroscopically There were practically no acid producing cells and the lining epithe lium of the glands was atrophic In the most atrophic areas the glands were decreased in number and lay too far apart — just as was observed in the intestines The lymphatic tissue was also very atrophic The granulation tissue however was also very scanty and was found in the greatest amounts in the most atrophic areas in the less atrophic parts of the mucosa only some large cells with hyperchromatic nuclei and a few cells presenting all the characteristics of Sternberg Reed cells lay scattered between the glands (fig 4) Hence it could be inferred that the process which had involved the stomach was basically similar to that involving the intestines There was no penetration of the muscularis mucosae submucosa muscularis and serosa were not affected

In the *duodenum* just outside the pylorus lymphogranulomatous tissue became much more abundant especially in the superficial layer of the mucosa Locally there was some penetration of the muscularis mucosae and a slight infiltration of the submucosa

The wall of the *common bile duct* was not affected up to the papilla Vateri the lymphogranulomatous tissue was strictly limited to the duodenal mucosa The gall bladder wall was also normal

In the *jejunum* too the muscularis mucosae appeared to be locally penetrated by the Hodgkin tissue some of the latter lay just underneath but the rest of the submucosa the muscularis and the serosa were free of Hodgkin tissue This held for the entire in testinal tract Even in the *valvula Bauhini* the process was limited to the (very atrophic) mucosa Sections of the *colon descendens* showed in addition to the Hodgkin tissue in the mucosa many neutrophils in the mucosa and submucosa

The *appendix* showed the same alteration as the rest of the gastrointestinal tract the mucosa was atrophic and contained typical Hodgkin tissue the glands were reduced in number and widely separated and the lymphatic tissue was extremely atrophic In fact it consisted only of remnants which contained moreover somewhat atypical reticular cells

As to the *mesenteric lymph nodes*, their con tours appeared to be preserved altough there was some lymphocytic and plasmocytic in filtration of the perinodal tissue in places Some lymph nodes showed a complete replacement of their normal structure by lymphogranulomatous tissue with atypical reticulum cells plasma cells lymphocytes eosinophils and slight fibrosis In others the general architecture of the node was *not* disturbed *in the sense* that broad sinuses locally showing some degree of fibrosis separated small patches of lymphatic tissue containing scattered Hodgkin cells and Sternberg Reed cells Locally the *normal structure* appeared to be wiped out by the typical granulomatous tissue One small necrotic area was seen

Lymph nodes from other sites of the body did not show any alterations suggestive of Hodgkin s disease The *spleen* showed areas with many neutrophils and a few scattered atypical giant cells (vesicular wrinkled nuclei with very poor chromatin) The *liver* did not show any sign of lymphogranu lomatosis in the portal areas only few lymphocytes were found The parenchymal cells however showed advanced atrophy and severe diffuse fatty degeneration There were small bile thrombi in places The *lungs* presented focal atelectasis and small broncho pneumonic areas in the lower lobes particu larly the right there was a fibrosis pleurae on either side *Trachea* and *br nchi* showed no signs of inflammation The *thyroid gland* presented somewhat enlarged follicles among normal sized ones The parathyroids were normal in appearance The *kidneys* and the other urogenital organs as well as the *adrenals*

the *pancreas*, and the *heart* showed a normal microscopical picture The *red bone marrow* contained haemopoietic tissue of a normal appearance

Results

A 46-year-old man had suffered since the spring of 1960 from attacks of diarrhoea, during the autumn of 1960 he developed a malabsorption syndrome and steatorrhoea, which appeared to be incurable The patient gradually became cachectic and developed a disturbance of liver function He slowly became icteric and died in March 1961 in coma Autopsy showed two alterations in the gastrointestinal tract a) lymphogranuloma malignum, localized diffusely in the mucosa throughout the gastrointestinal tract, from the stomach down to the rectum, and in the mesenteric lymph nodes, and b) extreme atrophy of the mucosa of the stomach and the small and large intestines

Discussion

According to the literature the lymphogranulomatous lesion may be limited to one or to a few isolated parts of the gastrointestinal tract (6, 7, 10, 11, 16, 17, 18, 20, 24, 25, 29, 30 40, 42, 45, 48), in other cases many lesions appear to be "disseminated" over the stomach, the small and large intestine (2, 3, 12, 15 33 37, 38, 44 46) Most authors agree that lesions of the stomach (1, 4, 25, 33, 38, 39, 42 46, 47) come first in the order of frequency, the antrum and prepyloric region in particular being favourite sites In the small intestine, the jejunum (7, 11, 17, 18, 21, 39, 44, 46, 47, 48) is the site of predilection, the terminal part of the ileum (15, 16, 24) — or the ileocoecal region — is often mentioned as the next favoured site, whereas the duodenum (10, 11, 29, 33, 38, 46, 47, 48) and the rest of the ileum (2, 3, 7, 12, 13, 15, 33, 37, 38, 44, 45) would seem to be involved less frequently (27, 32) Lesions of the large intestine (6, 14, 15, 19, 20, 29, 30, 33, 40, 46, 48) are generally considered to be rarer and to occur more frequently in the caecum (6, 20, 29, 30) and in the rectum (14, 19, 38, 40, 46) than in the rest of the colon (15, 29, 33), where isolated lesions in particular (29) have seldom been described Involvement of the appendix (23, 30, 32, 46) is also rare Cases have been described in which the lesion was localized at the ileocoecal valve (6, 20, 29) The most infrequent lesions are those of the tongue, tonsils, pharynx, and oesophagus (23, 26, 32, 33, 46) Craver and Herrmann reviewed the literature in 1946 and stated that intestinal Hodgkin's disease is most frequently localized in the proximal part of the small intestine and that its frequency decreases from there towards the rectum In the present study, 112 case reports of patients with lesions confined to, or mainly localized in, the gastrointestinal tract, were collected from the literature Lesions were found in the stomach in 51, in the duodenum in 20 in the jejunum in 35, in the ileum in 25, in the terminal ileum in 15, in the caecum in 8, in the appendix in 3 in the rest of the colon in 14 and in the rectum in 9 patients

The lymphogranulomatous lesions may show different macroscopical as-

pects A "tumour type" and an "ulcus type" of lesion can be distinguished The "tumour type" may either show a circumscribed polypoid growth into the lumen or a more or less diffuse infiltrative growth in the intestinal wall over a certain distance Combinations of these types of lesion in one specimen are often found In the polypoid form single or multiple tumour masses may be found varying in size from a pin head to an orange they may cause ileus by obstruction of the lumen (17, 18, 20, 29, 48) whereas the infiltrative growth may give rise to thick and rigid plicae (resembling cerebral gyri (38)) or to an intestinal segment entirely converted into a rigid tube (21), ileus caused by cicatrization in this type of growth seems to be rare (12, 26) Both types often show erosion or ulceration If the lesion is situated in (29) or near (24, 30) the ileocoecal valve invagination of this part of the intestine into the ascending colon may occur Cases of jejunojejunal intussusception have also been described (18 21) The "ulcus type" may be encountered when necrosis has been predominant from the beginning The ulcus usually shows elevated, peripherally sloping walls and a base covered with smeary sticky material (12 27) In this type of lesion either erosion of a blood vessel leading to intestinal haemorrhage (1 38 44) or perforation of the intestinal wall leading to peritonitis (7 12 30 46 47) may occur

Apart from the complications outlined above profuse diarrhoea (sometimes bloody) has been described as the most constant clinical symptom of intestinal lymphogranuloma (12 15 39) It may occur in periods, or alternating with constipation, and in various grades of severity Only in a few cases is mention made of steatorrhoea with secondary deficiency symptoms (2, 3, 12 13, 37, 41) The latter condition would follow obstruction of the chyle vessels by a disease process in the mesenteric lymph nodes (5, 36) The alterations in the mucosa may play a role as well (12), while impaired vitamin resorption may reinforce the functional impairment of the resorbing epithelium (2) It seems from the pathological reports of those cases of intestinal Hodgkin's disease in which the patient had suffered from steatorrhoea, that often there were multiple lesions disseminated over the entire small intestine (2, 3, 12) or a large part of it (37) The diarrhoea frequently is associated with abdominal pain, and may rapidly lead to progressive weight loss, prostration and anaemia — sometimes combined with leukopenia (40) or eosinophilia (29 44) Often there is fever, sometimes continuous, sometimes irregular (Pel Ebstein type) In some cases pruritus has been described (12, 18, 29)

Some authors stress the abdominal pain as the main complaint (7), this may apply particularly to epigastric pain in Hodgkin's disease of the stomach (25) Alcohol intolerance has been mentioned as a symptom of intestinal Hodgkin (7)

In most cases the right diagnosis was made only at laparotomy or at autopsy

There is no agreement among different authors about the exact site of primary localization of the lymphogranulomatous process in the intestinal wall According to many (6 15 39, 40) it

originates in the submucosa, as it may be entirely limited to, or have its greatest extent in, that layer Others describe the process as starting in the mucosa (9, 16), some writers take the mucosa and the submucosa together as the usual site of origin of the disease (12, 26, 30), which would primarily involve the lymph follicles and their aggregates, the patches of Peyer (20, 27)

Lymphogranuloma malignum has always been considered as a disease primarily involving lymphatic tissue (43), and lymphatic hyperplasia as the first alteration in this disease (22, 34, 35, 39) The proliferation results in the appearance of the characteristic large cells and giant cells (34) Lymphatic tissue is chiefly found within the mucosa, though normally, some of the greater lymph follicles or their aggregates may extend through the muscularis mucosae into the submucosa Thus, the way to spreading in the submucosa is open" (12) A few authors, however, describe cases of intestinal Hodgkin in which the process is localized outside the lymphatic tissue, leaving it (and especially the *lymph follicles*) *unaltered* (15, 38) It has been stated that the lymphoid system in the stomach is occasionally, and that of the small intestine almost never involved (35), but that, instead, the lymphoid tissue in the intestine undergoes atrophy (9, 35)

Histologically the tissues of the mucosa and/or submucosa, are replaced by Hodgkin tissue', the descriptions of which usually correspond with the granulomatous form of the disease outlined by Jackson and Parker in 1947 (23) The malignant granulomatous tissue usually extends through the thickness of the mucosa and the submucosa, and may infiltrate the muscularis, according to some authors (12, 15, 27) the longitudinal muscle layer seems to offer more resistance than the circular layer but the malignant cells may, nevertheless, reach the serosa From the intestinal wall the disease may spread to the mesenteric lymph nodes, which are usually involved but may (at least initially) remain free (7, 12, 38, 40, 47) According to some authors, the reverse may also take place primary involvement of the mesenteric lymph nodes (14, 19) and retrogressive spread to the intestinal wall

The lymphatic channels sometimes become obstructed by the process in the lymph nodes which may result in dilatation of these vessels and in chylus congestion (varices lymphatici intestini' (5)) This can be observed both macroscopically (chylus vessels lying as thick bands on the intestine) and microscopically (dilated lymph vessels in the villi and in the submucosa, sometimes even cyst like (oil cysts")) This would (perhaps together with the alterations in the mucosa itself (21)) result in severe steatorrhoea, which event has been described in some cases (2, 3 12, 13 37) These cases were, together with other forms of intestinal lymphoma, reviewed by Sleisenger et al in 1953 (41)

Our patient differs from the patients with gastrointestinal Hodgkin thus far encountered in the literature firstly in that the disease process was localized *diffusely throughout the stomach the small and the large intestine* instead of manifesting itself in one or more lesions or in *multiple*, disseminated lesions, secondly in that

there were no ulcers and no tumours, not even the slightest elevation of the mucosa or thickening of the intestinal wall, and, thirdly, in that the process was almost entirely limited to the mucosa. Lastly, the mucosa was extremely atrophic throughout the entire small intestine: no villi and only thin plicae were left, the mucosa of the large intestine, and even of the appendix, was very thin as well. The lymphatic tissue (follicles and patches of Peyer) was moreover scanty and very atrophic throughout the gastro-intestinal tract.

In an introduction to a symposium on the cellular elements of Hodgkin's granuloma and their normal functions (35), Rottino writes that in Hodgkin's disease of the small intestine the lymphoid tissue is almost never specifically involved, but that it does undergo atrophy. Some authors describe the lymphoid tissue in their cases as remaining *unaltered* (15, 38) or partially unaltered (46). In our patient, the relationship between the atrophy of the mucosa as a whole (apart from the lymphoid atrophy) and the presence therein of the lymphogranuloma is not exactly clear, although it seems conceivable that the atrophy is somehow secondary to the Hodgkin process. The severe steatorrhoea must be explained on the basis of the atrophy (and of the presence of the lymphogranulomatous tissue itself?), since there were no dilated chyle vessels indicating any chyle obstruction.

It is difficult to say what influence the administration of mitoxin and the irradiation of the abdomen may have had on the disease process. Macroscopically, neither the intestines nor the mesenteric lymph nodes differed at autopsy in any respect from their appearance at laparotomy. Microscopically, the mesenteric lymph nodes removed at autopsy showed somewhat fewer atypical reticulum cells than those excised at laparotomy.

As regards the jaundice developed by our patient in the final stage of his illness, we can only say that there were signs of haemolysis as well as of bile obstruction, but that the latter could not be confirmed at autopsy.

Summary

A short review is given of the literature on gastrointestinal Hodgkin's disease. A patient is described who suffered from profuse and incurable steatorrhoea; biopsy of a mesenteric lymph node revealed the underlying process to be lymphogranuloma malignum Hodgkin. The course ran progressively downhill. At autopsy lymphogranulomatous tissue was found to be diffuse in the mucosa throughout the gastrointestinal tract, from the cardia of the stomach down to the rectum, and in the mesenteric lymph nodes, but in no other organs. In addition, extreme atrophy of the mucosa of the stomach and of both small and large *intestine* was found.

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Latent Parathyroid Insufficiency Following Thyroidectomy

II Effect of Intravenous Infusion of Calcium upon the Serum Calcium

By

S Hahnemann and Th Friis

In recent years several reports have been published on latent parathyroid insufficiency in thyroidectomized patients (3 8 9) As a diagnostic criterion the authors have used a fall in serum calcium to subnormal values during a tolerance test with sodium phytate In a previous paper (5) the present authors reported such a decrease in 5 out of 10 thyroidectomized patients showing no clinical signs of hypoparathyroidism and having normal serum calcium levels being no corresponding decrease in 10 controls

The function which in these investigations forms the basis for assessing the integrity of the parathyroid glands is the calcium mobilizing capacity (by way of an increased bone absorption) which is assumed to be central mechanism in the parathyroid regulation of serum calcium

However recent studies have furnished experimental evidence for the presence of 2 hormones in the parathyroid glands (2, 7) Evidently one of these hormones possesses the stated calcium mobilizing capacity The production of this hormone is increased in response to a negative calcium balance The other hormone (which Copp et al (2) have designated calcitonin) will be produced in increased quantities in response to a positive calcium balance and counteract an increase in the serum calcium level Jointly the function of the two hormones is balanced against each other thus keeping the serum calcium level under normal conditions very close to constant Baghdianty et al (1) have succeeded in isolating a polypeptide with a calcitonin like effect from hog thyroid It has not yet been elucidated where the calcitonin is formed

Accordingly we felt that it would be of interest to investigate alterations in the serum calcium level after an induced positive calcium balance (in this case

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TABLE III Effect of calcium infusion (15 mg/kg) *in* 14 thyro dectomized patients without manifest parathyroid insufficiency Cases 1 7 and 8 had latent parathyroid insufficiency (the minimum serum calcium during the phytate tolerance test be ng < 4 50 mEq/l) The un derlined values indicate that the calcium level was > 5 5 mEq/l 20 hours after the infu sion

Case	Serum calcium prior to infusion (4 5-5 5 mEq/l)	Difference between serum calcium level after infusion and initial level		Maximum fall during phytate tolerance test level compared with pre test	Minimum serum calcium during phytate tolerance[1] test
		4 hours	20 hours		
1	5 00	+ 1 50	+ 0 70	0 30	4 30
2	5 40	+ 1 60	+ 0 60	0 40	5 00
3	5 15	+ 1 50	+ 0 60	0 50	4 65
4	5 00	—	+ 0 80	0 40	4 80
5	5 35	+ 1 30	+ 0 30	0 45	4 70
6	5 00	+ 0 90	— 0 30	0 25	4 50
7	4 75	+ 0 95	+ 0 05	0 35	4 30
8	5 45	+ 0 95	— 0 05	0 65	4 20
9	5 00	+ 0 70	+ 0 10	0 20	4 80
10	4 80	+ 0 40	— 0 00	0 10	4 70
11	4 80	+ 0 90	+ 0 40	—	—
12	4 70	+ 0 90	0	0 45	4 50
13	5 20	+ 0 65	— 0 20	—	—
14	5 30	+ 0 75	0	—	—
m	5 06 ± 0 250	+ 1 00 ± 0 370	0 29 ± 0 359		

[1] Minimum values during phytate tolerance test are stated since the *initial levels* were not always identical with the initial levels prior to *calcium infusion*

in the form of calcium gluconate infused over 4 hours (from 9 a m to 1 p m) in 1 2 l physiological saline Blood samples for serum calcium determination were obtained prior to the infusion and 4 and 20 hours after the end of the infusion the first and the last after at least 8 hours fasting

Serum calcium was determined by the murexide EDTA method (4) The *normal* range in our laboratory proved to vary between 4 5 and 5 5 mEq/l

Results

For assessing the results we used the difference between the serum calcium concentration 4 or 20 hours after the end of calcium infusion and the initial s *rum calcium level*

The results are given in fig 1 and in tables I—III

1 *Ten normals*

As is evident from table I, the *serum* calcium level in these patients had at 20 hours after the end of the *infusion* fallen down to or almost down to the initial level The difference ranged from − 0 10 to + 0 35 mEq/l, *m* = + 0 15 mEq/l The corresponding difference between the 4 hour values and the initial values was between + 0 60

TABLE I Effect of calcium infusion (15 mg/kg) in 10 non thyroidectomized patients

Case	Serum calcium prior to infusion (4 5—5 5 mEq/l)	Difference between serum calcium level after infusion and initial level	
		4 hours	20 hours
1	5 40	+0 60	—0 10
2	4 95	+0 65	+0 15
3	4 60	+0 70	+0 30
4	5 10	+0 70	+0 10
5	5 00	+0 65	+0 35
6	5 00	+0 30	0
7	4 90	+1 40	+0 10
8	4 60	+0 80	+0 10
9	4 80	+0 90	0
10	4 80	+1 70	+0 25
Mean	4 92±0 238	+0 84±0 410	0 15±0 119

TABLE II Effect of calcium infusion (15 mg/kg) in 2 patients with manifest parathyroid insufficiency Case 2 had been thyroidectomized

Case	Serum calcium prior to infusion (4 5-5 5 mEq/l)	Difference between serum calcium level after infusion and initial level		Maximum fall during phytate tolerance test level compared with pre test	Minimum serum calcium during phytate tolerance test
		4 hours	20 hours		
1	4 20	+1 50	+0 90	0 60	3 60
2	4 30	+1 65	+1 00	1 20	2 60

intravenous infusion of calcium) in thyroidectomized patients compared with the response in normals

Material

Ten patients with no signs of endocrine disorders or disturbances of the calcium phosphorus metabolism (table I)

Two patients with manifest hypoparathyroidism idiopathic in one following thyroidectomy in the other (table II)

Fourteen unselected euthyroid patients who had previously undergone subtotal thyroidectomy (8 for toxic and 6 for non toxic goitre) in 1937—1963 All had normal serum calcium and showed no signs of manifest parathyroid insufficiency (table III)

Methods

The sodium phytate tolerance test was carried out as previously described (5) by oral administration of 9 g daily for 3 days The tolerance test was performed on 11 of the 14 thyroidectomized patients and on the 2 patients with manifest hypoparathyroidism

For the intravenous infusion of calcium 15 mg calcium per kg body weight was used,

This observation may support the idea that there are two calcium regulating hormones and indicate that thyroidectomy may cause isolated deficiency of either hormone

A comparison of the control group and the thyroidectomized group without manifest parathyroid insufficiency shows that the mean values for the difference between the initial level and the 20 hour level are + 0 15 and + 0 29 mEq/l, and those for the difference between the initial level and the 4 hour level + 0 84 and + 1 00 mEq/l The values do not show a statistically significant difference In general, the values in the thyroidectomized patients showed a greater standard deviation than those of the normals

It is concluded that in some cases thyroidectomy may cause disregulation of serum calcium the latter being more markedly changed through alterations in the calcium balance than in normal subjects

Summary

In 10 patients without endocrine disorders or disturbances of calcium phosphorus metabolism in 2 with hypoparathyroidism and in 14 thyroidectomized patients having normal serum calcium the serum calcium level was determined before as well as 4 and 20 hours after an intravenous infusion of calcium (15 mg kg, during a 4 hour period) While none of the controls had a 20-hour value exceeding the initial level by more than 0 35 mEq/l, 5 of the thyroidectomized patients showed a greater difference Five of the thyroidectomized patients, unlike the controls, showed a serum calcium, 20 hours after the infusion, above the upper limit of the analytical range (5 5 mEq/l) The 2 patients with manifest parathyroid insufficiency also showed a marked elevation of serum calcium 20 hours after the infusion, as compared with the initial level

Only one of the patients having an elevated serum calcium 20 hours after the infusion was suffering from latent parathyroid insufficiency as assessed by the sodium phytate tolerance test The assumption is advanced that thyroidectomy may entail a defect in the output of the anti parathyroid hormone calcitonin described by Copp et al

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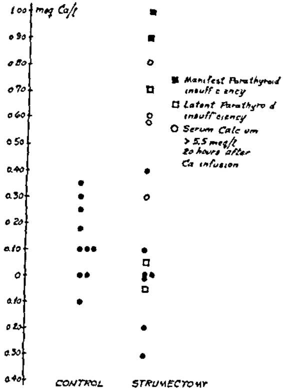


Fig 1 Difference between serum calcium levels before and 20 hours after infusion of calcium (15 mg/kg)

and + 1 70 mEq/l, m = + 0 84 mEq/l In all cases the serum calcium level 20 hours after the end of the infusion was within the normal range A similar finding has been reported by Howard et al (6)

B Two patients with manifest parathyroid insufficiency (table II)

In these two patients the serum calcium level 20 hours after the end of the infusion was considerably above the initial level A similar finding has been reported by Howard et al (6)

C Fourteen thyroidectomized patients without manifest parathyroid insufficiency (table III)

The serum calcium level 20 hours after the end of the infusion was more than 0 35 mEq/l above the initial value in 5 patients (the greatest increase found in the control series) (table III, cases 1—4, 11) In 5 patients the absolute level of serum calcium was still above 5 5 mEq/l 20 hours after the infusion was completed (table III, cases 1—5) Their serum calcium levels prior to the infusion had not differed definitely from that of the others All these 5 patients were submitted to sodium phytate tolerance, but only one showed latent parathyroid insufficiency (case 1), the serum calcium level dropping to below 4 5 mEq/l during the test Of the remaining 9 patients, 6 were investigated by phytate tolerance Two showed latent parathyroid insufficiency (cases 7 and 8) In respect of the 4 hour values the difference between the two groups of patients is less striking, as none of the thyroidectomized patients increased by more than 1 70 mEq//l, the greatest increase exhibited by the normal group At 4 hours all were above the upper limit of normal for the analysis, except 3 of the control group and one of the thyroidectomized patients

Discussion and conclusion

Accordingly, there seems to be a defect in the regulation of the serum calcium level in some thyroidectomized patients, measured not only by the response to an induced negative calcium balance (phytate tolerance), but also by the reaction to calcium infusion An abnormal response to a phytate tolerance test apparently does not necessarily entail an abnormal reaction to calcium infusion, or vice versa

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Simultaneous Occurrence of Two Immunologically Different M-Components in Serum

By

ROLF BACHMANN

Multiple M components[1] have been demonstrated in one and the same patient by paper electrophoresis (18, 22), starch gel electrophoresis (8 19) immunoelectrophoresis (14) and ultracentrifugation (7 8 16) M components of immunologic type γ_μ in the serum and/or urine often occur in association with serum M-components of type γG γA or γM (22) The relationship between γ_μ globulins and other immunoglobulins has not been fully cleared up Observations made by Askonas and Fahey (2) however suggest that both γ_μ and γG M components are produced by one and the same mouse plasma cell tumour i e by cells of one and the same genetically stable cell line The occurrence of multiple γA or γM M components can as a rule be explained by polymerisation (4, 7, 8) *or complex* formation with normally occurring proteins such as albumin (4 14) Starch gel electrophoresis has revealed a heterogeneity of γG M *components*, which is not due to polymerisation and whose nature is still obscure (8, 11) No antigenic differences have been demonstrated among the different fractions A mouse plasma cell tumour that produced γG M-components with such heterogeneity showed no change on transplantation (1)

Multiple M components whose characteristics suggest that they are produced from genetically different stable cell lines have been reported in single cases Harboe et al (12) have described one case with two γA M-components and another case with one γA and one γ_μ M-component In both cases the M-components had different InV genotypic specificity Fine and Creyssel (10) reported one case, and Debray Sachs (6) three cases of myelomatosis

[1] Immunologically characterized globulins fall into the following subgroups γ globulins = γG [illegible] 7S [illegible] β₂A-globulins = γ1A = [illegible] β₂M-globulins γ1M 19S = γM γ_μ = [illegible] micromolecular type so-called Bence Jones proteins.

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Results

Paper electrophoresis of the seven cases are shown in fig 1 Only in two cases (770 913) were the two M components easily observed In the other cases one M component was quite distinct and the other could only be suspected from the paper electrophoretic pattern In case 647 the two M components had nearly the same paper electrophoretic mobility

Immunoelectrophoresis against specific rabbit anti γG globulin and anti γA globulin antiserum of the seven cases invariably showed an atypical bow on both the γG globulin and the γA globulin line Immunoelectrophoresis with the other antisera (specific rabbit anti γM globulin rabbit anti immunoglobulin and horse antihuman antisera) verified the findings The immunoelectrophoretic patterns of two of the cases (770 826) are shown in fig 2

In three of the cases (770 826 913) the M components were purified by ammonium sulphate precipitation preparative electrophoresis and column chromatography Paper electrophoresis starch gel electrophoresis and immunoelectrophoresis specific rabbit anti γG globulin and anti γA globulin and rabbit anti immunoglobulin antiserum) of the purified M components verified the aforementioned findings Immunoelectrophoresis of normal serum to which one of the purified M components had been added showed the atypical bow of the added M component but not of the other Treatment of the purified M components with 0 1 M mercaptoethanol before immunoelectrophoresis produced no change in the immunoelectrophoretic pattern

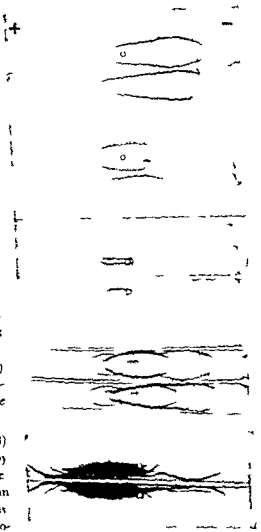


Fig 2 The immunoelectrophoresis in case No 770 (the upper on each slide) and case No 826 the lower on each slide The antisera used are from above specific rabbit anti γG globulin antiserum specific rabbit anti γA-globulin antiserum specific rabbit anti γM-globulin antiserum rabbit anti immunoglobulin antiserum and horse anti human antiserum

In table I the age sex and clinical diagnosis of the patients are given together with the paper-electrophoretic



Fig. 1. The paper electrophoretic strips of the seven cases with co-existing M components of type γG and γA. The arrows indicate the M components.

with double M components, one of type γA and one of type γG. Kjeldsen and Asfeldt (15) have described a case with double M components, one of which was of type γG and the other of type γM. In one and the same mouse Potter and Kuff (25) succeeded in inducing plasma cell tumours which produced M components with electrophoretic and immunologic differences and which retained their individuality on transplantation.

As judged from these reports of sporadic cases only rarely is there simultaneous occurrence of multiple M components with characteristics suggesting that they are produced by multiple cell lines. This assumption is, however, not corroborated by experience in our laboratory, where we have found 7 cases with co-existing γA- and γG-M-components which suggests that a thorough analysis of the antigenic structure in *cases with multiple M components of* one and the same type would have revealed more cases with double M components with antigenic differences that could be explained by their production of different cell lines.

Material

Of the 870 sera with M-components studied at our laboratory, the last 585 were examined with the same set of antisera. Of these 585 sera seven (569, 647, 703, 749, 770, 826, 913) were found to contain two M components *of immunologically different* types (γG and γA).

Methods

The methods for electrophoretic and immunologic classification of M-components in serum have been described elsewhere (3).

Preparative electrophoresis with Pevicon as supporting medium was done according to Müller-Eberhard (20).

Chromatography on DEAE-cellulosa was performed with a continuous gradient of phosphate buffer with decreasing pH and increasing ionic strength.

Since the two M components in the seven cases differed in immunological type, it is reasonable to suppose that they were produced by genetically different cell lines

The frequencies of different types of M components (3, 5, 23, 28) correspond roughly to the normal relative concentrations of the different types of immunoglobulins (9 13) This suggests that different types of immunoglobulin producing cell lines have roughly the same tendency to undergo abnormal changes with consequent production of M components Viewed in this light the number of cases found with two M-components of different immunologic type was remarkably large This should mean that our material included a larger number of cases with double M components of the same immunologic type particularly type γG which could also have been produced by different cell lines But the possibilities of checking the validity of this assumption are limited especially when the concentration of the M components is low

The material included 119 sera with *one* M component of immunologic type γA These included the 7 cases with an additional M-component of type γG It is also possible that one or more of the remaining 112 sera contained two M components of immunologic type γA that might have been produced by different cell lines These figures indicate that in 5 10 of the cases with M components there was an abnormal M component production by more than one cell line In only 1 913 of the 7 cases was the occurrence of double M components known before our electrophoretic examination It is therefore unlikely that an unintended selection of the sera sent to our laboratory explains the rather high frequency in our material It is remarkable that we have not yet found any cases of double M components of different immunological type where the one was of type γM Conceivably we may miss some M-components of type γM in low concentration, because about half of these do not penetrate the agar gel without preceding cysteine treatment which was done only for special indications

In some breeds of mice certain agents will induce M component producing plasma cell tumours in high frequency In investigations of one of these mouse strains Potter (26) found that these stimuli could produce a variety of different plasma cell tumours in one and the same animal and that these tumours produced different M components and could be transplanted separately Later, however, only one type of plasma cell tumour could be demonstrated This would suggest that a number of plasma cell tumours can be induced simultaneously, but that one of them gradually ousts the others Whether this also holds for man is not known In case 770, which was followed for nine months both M components could be demonstrated every time On the other hand it should be observed that the concentration of one of the M-components was low (< 0.5 g/100 ml) in six of the seven cases

It should be mentioned that in three cases without signs of myelomatosis the patients had or had had cancer and one also reticulum-cell sarcoma The occurrence of M-components has been

TABLE I The estimated serum concentration and paper-electrophoretic mobility of the M-components, sex, age and clinical diagnoses in the seven cases with simultaneous occurrence of M components of type γG and γA

Case No	Sex	Age	γA—M—component: Concentration (g/100 ml)	γA—M—component: Paper-electrophoretic mobility	γG—M—component: Concentration (g/100 ml)	γG—M—component: Paper-electrophoretic mobility	Clinical diagnosis
569	♀	68	0 1	β_2	0 5	γ_{1-2}	Colon carcinoma op 2 yrs before the serum electrophoresis No known metastases
647		69	0 5	β_2	0 4	β_2–γ_1	Undifferentiated carcinoma with metastases
703	♀	54	0 1	β_2	6 1	γ_{1-2}	Myelomatosis
749	♂	69	0 1	β_2–γ_1	0 4	γ_{1-2}	Reticulum-cell sarcoma + renal carcinoma
770	♀	70	0 4	β_{1-2}	3 1	γ_1	Myelomatosis
826	♂	65	0 2	β_2	3 3	γ_2	Myelomatosis
913	♀	66	1 3	β_{1-2}	3 7	γ_1	Myelomatosis

mobility and the estimated concentration of the M components The diagnosis of myelomatosis was based on the bone marrow picture and/or skeletal X-ray The other diagnoses were verified by biopsy and/or at necropsy All the patients had malignant diseases (myelomatosis in four carcinoma in two reticulum cell sarcoma and carcinoma in one)

Sera were obtained from one patient (770) six times within nine months Paper electrophoresis always showed both M components

Discussion

That the M components really were of different immunologic types is apparent already from the results of the immunoelectrophoresis with specific antisera against γG- and γA globulins, and is supported further by the results of the other examinations If there had been only one M component, of type γG or γA with a tendency to complex formation with other normally occurring immunoglobulins both the M component and the complexes would have been observed on immunoelectrophoresis with either specific anti γG globulin or anti γA globulin antiserum Addition of purified M components separately from three of the cases to normal serum resulted in the development of only one M component (the one added) which also argues against complex formation being responsible for the double M components

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reported in patients with cancer (17, 23, 29, 30), but it is not known whether there is any relationship between the cancer disease and the simultaneous occurrence of M components One might imagine that certain tumour-inducing agents can also induce proliferation of abnormal plasma cells with the formation of M-components as a consequence The occurrence of double M components and carcinoma or sarcoma could then be interpreted as a manifestation of exposure to a heavy dose of such stimuli

Summary

Of 585 sera with M components studied electrophoretically and immunologically, 7 were found to contain two M components of different immunologic type In all the 7 cases one of the M-components was of type γG and the other of type γA In no instance of multiple M components was one of them of type γM

Since the material included 119 sera with M-components of immunologic type γA it may be assumed that the frequency of multiple M components produced by genetically different cell lines is as high as 5—10 % among the sera with M components

In 3 of the cases the M components were separated and purified by precipitation, preparative electrophoresis and column chromatography

The clinical diagnoses in the 7 cases were myelomatosis in four, carcinoma in two, and carcinoma and sarcoma in one

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From the Renal Ward Fourth Medical Clinic University of Helsingfors Finland

Glomerulonephritis with Initial Pulmonary Haemorrhage

By

A. PASTERNACK, E. LINDER and B. KUHLBÄCK

A syndrome consisting of initial pulmonary haemorrhage with glomerulonephritis was first described by Goodpasture in 1919 (4). Since then several reports have appeared dealing with cases of haemoptysis followed by severe renal disease rapidly progressing to renal failure and death (1, 2, 5, 9, 10, 12–16). Few patients are known to have survived the acute attack (1, 2, 6, 15, 17, 20). A surviving patient with this rare syndrome is reported.

Case report

A women aged 43 was admitted to the Renal Ward on May 15, 1964 because of oliguria and uraemia. She had a history of tetanus at 17 years of age and several attacks of tonsillitis. Her first attack of haemoptysis was in July 1963 when it followed otitis media. No clinically obvious signs of impaired kidney function were noticed. After rapid resolution of that attack the patient had remained in good health until April 1964 when she had an upper respiratory infection followed by impaired general condition and haemoptysis. The patient was admitted to a chest ward on April [illegible] 1964.

On her admission to the chest ward the urine contained numerous erythrocytes but no protein. Hb 9.5 g/100 ml, white cell count 7800/mm³ with 3 % eosinophils. The ESR was 67 mm 1 h. Bleeding and coagulation times were normal. Serum creatinine was 0.8 mg/100 ml. The chest X-rays showed diffuse opacities in the central and lower lung fields. For 10 days the patient was treated with penicillin (procaine penicillin 1 800 000 I.U. daily) and for another 10 days with tetracycline (Rondomycin® 600 mg daily). During that time a gradual deterioration of the general condition took place. The blood stained sputa continued and protein appeared in the urine. The anaemia became more pronounced. The urinary output was not measured but it was considered normal. The patient became uraemic and was admitted to the Renal Ward.

On her admission serum creatinine was 10.8 mg/100 ml, Hb 7.9 g/100 ml, red cells 2 470 000/mm³, ESR 98 mm 1 h. No signs of haemolysis. B.P. 130/80 mm Hg. Chest X-rays showed the same opacities as before though they were less marked. After the day of admission polyuria developed with subsequent decrease of the NPN values and disappearance of the uraemic symptoms. The patient remained in the ward for one month.

Throughout this time the urinary sediment

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Case report

A women aged 43 was admitted to the Renal Ward on May 15 1964 because of oliguria and uraemia She had a history of tetanus at 12 years of age and several attacks of tonsillitis Her first attack of haemoptysis was in July 1963 when it followed otitis media No clinically obvious signs of impaired kidney function were noticed After rapid resolution of that attack the patient had remained in good health until April 1964 when she had an upper respiratory infection followed by impaired general condition and haemoptysis The patient was admitted to a chest ward on April 21 1964

On her admission to the chest ward the urine contained numerous erythrocytes but no protein Hb 9 5 g/100 ml white cell count 7800/mm³ with 3 % eosinophils The ESR was 67 mm/1 h Bleeding and coagulation times were normal Serum creatinine was 0 8 mg/100 ml The chest X rays showed diffuse opacities in the central and lower lung fields For 10 days the patient was treated with penicillin (procaine penicillin 1 800 000 I U daily) and for another 10 days with tetracycline (Rondomycin® 600 mg daily) During that time a gradual deterioration of the general condition took place The blood-stained sputa continued and protein appeared in the urine The anaemia became more pronounced The urinary output was not measured but it was considered normal The patient became uraemic and was admitted to the Renal Ward

On her admission serum creatinine was 10 8 mg/100 ml Hb 7 9/100 ml red cells 2 470 000/mm ESR 98 mm/1 h No signs of haemolysis B P 130/80 mm Hg Chest X rays showed the same opacities as before though they were less marked After the day of admission polyuria developed with subsequent decrease of the NPN values and disappearance of the uraemic symptoms The patient remained in the ward for one month Throughout this time the urinary sediment

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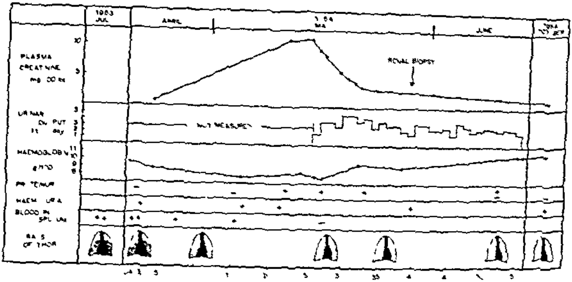


Fig 1 The course of the disease

contained numerous erythrocytes The degree of proteinuria decreased and was scarcely detectable at the end of the treatment period Serum creatinine decreased to 1 65 mg/100 ml, the endogenous creatinine clearance was 39 ml min ASТ was 45 and 180 The patient was given procaine penicillin 1,200 000 I U daily during one week and chloramphenicol 1 g daily for another week No corticosteroids were administered

The chest X-rays had cleared during this treatment period and on the patient's discharge from the hospital only scanty changes in the lower lung fields were detectable

Renal biopsy was performed on May 29 and showed capsular adhesions and increased collagenous tissue in the glomeruli One glomerulus was completely hyalinized and was surrounded by a cellular infiltrate In some of the glomeruli the damage was focal

At subsequent *out patient follow up* the patient has been well except for a period when she was given Doctacillin® for her urinary infection She then developed migrating red and tender swellings at the knees ankles fingers and left ear At the same time *the ESR increased* and the proteinuria and haematuria became aggravated When the treatment with Doctacillin was discontinued the symptoms immediately cleared up

Immunological studies

LE-cells were looked for repeatedly but were found on only one occasion DNA antibodies were not found when sought according to the method of Lawlis (8) No deoxyribonucleoprotein antibodies were found by the method of Friou (3)

The patient s serum was tested for the presence of circulating antibodies against human kidney and lung The two methods used were the gel diffusion technique according to Wadsworth (19) and an indirect immunofluorescence staining (11)

The gel diffusion was performed using whole kidney and whole lung homogenates as antigens and testing them against the patient s serum The result is schematically illustrated in fig 4 The precipitate lines did not develop if the patient s serum was absorbed with kidney homogenate before gel diffusion

The indirect fluorescent antibody test was performed using kidney and lung tissue from individuals without known disease in these organs Two different kidneys were used the one being frozen to — 20 C and cut frozen the other being freeze-dried and embedded in paraffin wax before cutting The lung tissue was frozen to — 20 C and cut frozen The cut sections were treated with the patient s serum at 37° C for a period of



Fig 2 Glomerulus from biopsy specimen showing severe slightly focal changes



Fig 3 Almost completely hyalinized glomerulus from biopsy specimen There is a moderate cellular reaction around the glomerulus.

four hours They were then rinsed in buffered saline for 10 min and treated with fluorescent goat anti human gammaglobulin (Difco) for 30 min The sections were rinsed for 10 min in buffered saline and mounted in Bacto FA mounting fluid The same procedures were followed with several sera from healthy persons as controls

Staining led to a stronger fluorescent reaction of kidney sections treated with the patient's serum than of those treated with the control sera The fluorescence was most pronounced on the basal membranes of the tubuli and on the cytoplasmic components of the kidney When a fivefold concentration of the serum was used in addition to this a nuclear fluorescence was observed On staining lung sections treated with the patient's serum gave no stronger fluorescence than those treated with the control sera

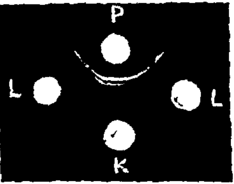


Fig 4 Precipitation pattern observed in gel diffusions using patient's serum (P) against homogenates of kidney (K) and lung (L) Identity reaction between kidney and lung can be seen There was also a slight precipitate against kidney that was not observed against lung

Discussion

In our patient the diagnosis of Goodpasture's syndrome is suggested by the fact that haemoptysis was the initial symptom preceding the signs of glomerulonephritis the first episode of haemoptysis taking place eight months before the second Furthermore the X rays showed a picture atypical of the pulmonary changes associated with uraemia and as in previously reported cases the anaemia was out of proportion to the apparent loss of blood

Periarteritis nodosa cannot easily be ruled out as a possible differential diagnosis As is pointed out by previous authors 20 there is a gradual change of the clinical pattern from cases



Fig 5 X ray of chest at beginning of hospitalization (A) and at the end of it (B)

presenting primarily as periarteritis nodosa to those presenting a pure "Goodpasture's syndrome" As is suggested by Lexow and Sigstad (9), the problem has so far to be considered more generally, "idiopathic pulmonary haemosiderosis" also being included in the same group of clinical manifestations

Hypersensitivity has been suggested to be the aetiological factor underlying this disease (13) Certain features present in our case also support this view These include the obvious delay in the appearance of kidney involvement after repeated pulmonary symptoms, the nature of the tissue reaction in the kidneys, which resembles glomerulonephritis of other origin, as well as the allergic manifestations during penicillin treatment that have been reported in several cases (12, 13)

Experimental studies (18) have revealed that anti lung serum operates as a nephrotoxic serum, producing nephritis, and localizes in the glomerular capillary walls Scheer and Grossman (16) suggested that Goodpasture's syndrome might be the human analogy of this animal experiment in the form of an autoimmune disease They showed a strong binding of gammaglobulin to the glomerular capillary walls in two patients with the syndrome Circulating anti bodies against human kidney were present in low titres in their patients

Demonstration of a precipitin reaction in gel diffusion between the patient's serum and extracts of kidney and lung in our case is not, of course, strong evidence for an autoimmune aetiology of the disease The identity of the precipitate line only suggests the presence of a circulating antibody against a common antigen in the kidney and lung The positive staining of normal kidney structures by the indirect fluorescent antibody technique is also in favour of a circulating anti kidney factor The results may reflect an aetiological mechanism of autoimmune nature but they might

also be secondary to tissue damage in both organs

The finding of an antinuclear factor is of special interest Supposing that it was not an unspecific finding due to physico-chemical factors, as described by Laffin et al (7), it might be linked with the positive LE cell phenomenon once observed in our patient This entitles us to believe that we are dealing with destruction of nuclear substance in the kidney leading to the production of an anti nuclear factor though this could not be demonstrated by the methods of Lawlis (8) and Friou (3) It provides no further support, however, for the supposed autoimmune aetiology of Goodpasture s syndrome

Concerning the aetiology of Goodpasture s syndrome it should be pointed out that a viral origin has been proposed (2) In our case this suggestion has to be seriously considered On both occasions of haemoptysis the patient had had initial symptoms of upper respiratory infection That an autoimmune mechanism operates later in the disease is still possible

The patient discussed in this paper presented an exceptionally benign course of Goodpasture s syndrome It has to be stressed that this was in spite of lack of any corticosteroid therapy Cases of patients surviving an attack of pulmonary haemorrhage glomerulonephritis will certainly accumulate when more attention is paid to the syndrome

Summary

A surviving patient with Goodpasture s syndrome (pulmonary haemorrhage with glomerulonephritis) is reported The aetiology is discussed in the light of immunological studies, revealing the presence of a circulating antibody against kidney and lung

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From the Department of Medicine (Head G Biorck M D) Karolinska Institutet at Serafimerlasarettet Stockholm, Sweden

Cardiotachygraphy — A Method for Continuous Long-term Recording of Heart Rate

By

LARS MOGENSEN and PALNE MOGENSEN

During recent years there has been increasing interest in extended observation of various indices of cardiovascular function Advances in the field of electronics have made possible not only recording and storage of such data but also if necessary its transmission over long distances through telemetering (6 9 10 11 15)

Continuous observation of the heart activity of patients is thus made possible both in natural surroundings and in artificial test situations varying from satellites to laboratories However the voluminous nature of the recordings so obtained makes analysis of the data impractical for more than a few special cases Means of compressing the information for more rapid yet accurate evaluation by the physician are therefore required The purpose of this report is to present a simple method whereby heart rate and its variations may be obtained on a vertical scale We have called this method cardiotachygraphy here after referred to as CTG

Submitted for publication December 28 1964

Methods

Ordinary ECGs contain information about frequency, although this is not immediately available as it would be in a frequency plot Furthermore, determination of heart rate from long term ECG recordings is laborious entailing storage of considerable masses of paper More important beat to-beat fluctuations in heart rate may easily be missed Tape recording is by far the most comprehensive means of storing the information and has inherent advantages for use in computors However for clinical application at present tape recording per se does not simplify heart rate determination

The duration of the RR interval in an electrocardiogram is inversely proportional to the heart rate RR intervals can be arranged and analysed electrically in fundamentally different ways Fig 1 A depicts the standard ECG representation with the RR intervals arranged in succession In fig 1 B the frequency of different durations is determined Finally fig 1 C illustrates vertical chronological placement

In the example B the time order is lost but information is obtained on the number of heart beats in each frequency class during the time of registration In the example C the connecting line between the upper points of the RR intervals will continuously record the

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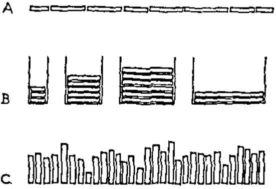


Fig 1 Different arrangements of RR intervals

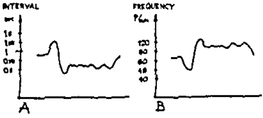


Fig 2 Heart rate, plotted as RR interval variations A Inversion yields a frequency plot, B

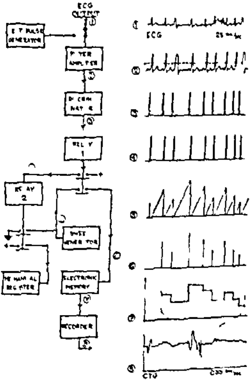


Fig 3 Working principle for the cardiotachygraph.

length of each, as in fig 2 A Inversion of the recording yields a record of heart rate, vertically increasing on an appropriate scale, fig 2 B This principle has been applied to the cardiotachygraph

Technical description

Fig 3 is a diagrammatic representation of the specific instrumentation employed

The output signal from the ECG apparatus, an Elema Schonander Mingograph, is taken in parallel with its galvanometer from a standard socket This signal often contains noise such as muscle potentials of high frequencies and base line drift caused by variations in electrode skin contact The frequency characteristics of these disturbances are generally different from those of the R wave, and are suppressed in a RC filter The signal is then amplified, taking the R waves up to an amplitude of approximately 2 volts The next step involves a discriminator through which only pulses greater than 1 volt are allowed to pass The R waves are then given a uniform shape and amplitude The time interval between successive R waves is converted into a proportional voltage through a sweep circuit, which increases voltage linearly with time This voltage is set to zero when the output is connected to ground by relay 2 When this relay opens, the voltage rises from zero again Relay 1 will close for a moment when the time interval between two R waves has elapsed The electronic memory will thus be set to the voltage generated in the sweep circuit Both relays have short closure time The voltage stored in the electronic memory will in this way be proportional to the time interval between the two latest R waves The memory delivers this voltage to the recording equipment As seen in fig 3 the relay 2 is controlled by relay 1 and is operated immediately after the drop of relay 1 This implies that as soon as the sweep voltage has been fed to the memory, the sweep is set to zero and its voltage rises again The resultant reading is somewhat distorted by the time constant of the needle motion in the

recorder Through special arrangements the pen speed has been increased so that separate RR intervals are easily visible The system involves a calibrator with a frequency range from zero to 600 cpm It consists of a saw tooth generator with a differentiating output circuit

The amplifier is a one step RC-coupled transistorized unit with a frequency range of approximately 5—20 cps The discriminator is a Schmitt trigger in which the output voltage can attain only two discrete values and switching occurs when a certain level is surpassed by the input voltage The sweep circuit consists of a bootstrap sweep with fast return The relays are monostable flip-flops with a closure time of approximately 20 ms The electronic memery is made up of a capacitator directly coupled to the grid of a cathode follower We have used a Siemens recorder with an input resistance of 126 ohms and max deflection of 10 mA The paper speed can be varied from 2 to 0 33 mm/s corresponding to 7 2 and 1 2 meter per hour respectively We have used inkpen and ordinary paper as well as *electric* pen and metallic paper each of which has inherent advantages and disadvantages

The sweep voltage rises linearly fig 4 and thus the RR intervals are reproduced on a linear scale On the other hand the frequency scale is exponential in nature Through adjustment of the time base however the output voltage could be made proportional to the frequency and linearity of the frequency scale thus obtained Thorough analysis on similar problems has recently been reported (19)

For operation the cardiotachygraph is connected to the oscilloscope output on an ordinary Mingograph An ECG lead is chosen that gives tall R waves and small or negative T waves A high R/T ratio prevents registration of the T waves Disturbances from muscle activity can imitate the R waves and be recorded as heart beats However certain leads contain little such disturbances and CM between manubrium and the fifth intercostal space on the left side has commonly been found appropriate

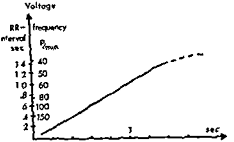


Fig 4 Relationship of RR intervals and frequency to sweep voltage

Extrasystoles are recorded when the complexes reach above the level of discrimination

An ECG may be obtained simultaneously with the cardiotachygram at any moment during the registration

The mechanical register makes it possible to follow the accumulated number of heart beats The number of beats per minute or per hour can thus easily be observed

Preliminary applications

The described instrument has been used in a pilot study on some different heart rhythms and a few representative recordings are shown in figs 5—13

a) The cardiotachygram from a young healthy subject at rest is shown in fig 5 The respiratory arrhythmia is clearly visible
b) Deep respiratory movements cause irregularities of the heart rate (fig 6)
c) In fig 7 an effect of psychic stress on the heart rate is demonstrated The patient was left alone in a room asked to stay in bed and the stress refers to telephone signals from a telephone out of reach of the patient
d) In atrial fibrillation the arrhythmia gives rise to recordings as in fig 8 the left half of which is recorded at rest the right half at exercise
e) In a case of supraventricular tachycardia pressure over one carotid sinus was performed and the resultant decrease in heart rate is shown in fig 9
f) The recording in fig 10 was obtained from a patient with nodal bigeminal rhythm

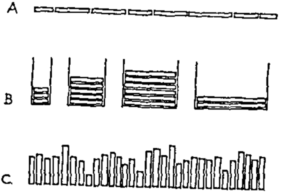


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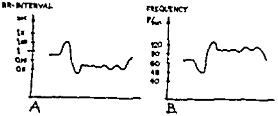


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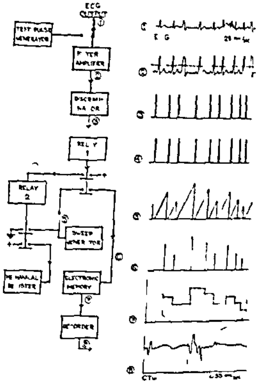


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The zigzag line indicates the two classes of RR intervals regularly occurring alternatively thus the difference between two successive intervals fairly regularly is around 0 04 seconds External influences alter the frequency without changing the bigeminal character This explains that the zigzag line changes level of frequency without change in shape The corresponding ECG is shown in fig 11

g) In a case of total AV block with isolated extrasystoles the recording looked as in fig 12 The basal heart rate is pathologically low and constant totally lacking the sinus arrhythmia found in healthy subjects The recording shows that the extrasystoles come at different distances from the preceding heart beat and that the first heart beats after each ES show a slight retardation before the earlier frequency is achieved

h) A patient was studied during treatment with two different agents called A and B in fig 13 She suffered from frequent extrasystoles, mostly in bigeminal or trigeminal form The cardiotachygram gives an idea of the number of extrasystoles during a comparatively long interval and thus enables a quantitative judgement on the effect of the different treatment procedures

PRESSURE ON CAROTID SINUS



Fig 9 Effect of vagal stimulation on supraventricular tachycardia



Fig 10 Nodal bigeminal rhythm cardiotachygram

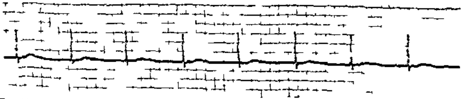


Fig 11 Nodal bigeminal rhythm electrocardiogram

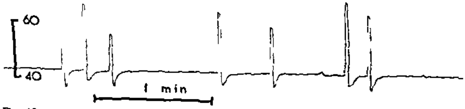


Fig 12 Idioventricular rhythm with isolated extrasystoles

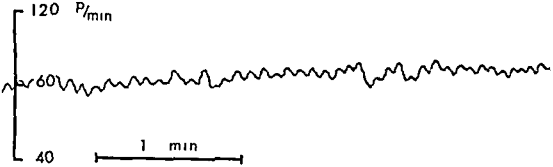


Fig 5 Heart rate variations with respiration



Fig 6 Heart rate variations caused by deep respiratory movements

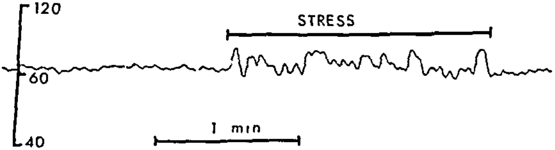


Fig 7 Effects of stress on heart rate

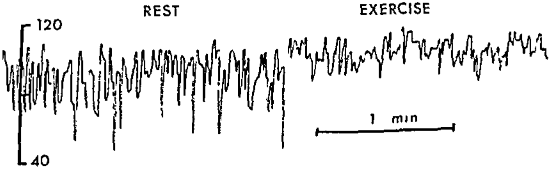


Fig 8 Arrhythmia due to atrial fibrillation at rest and during exercise

the heart rate A mechanical register gives the accumulated number of heart beats

The described equipment gives compressed information on heart rates as well as reliable information on the time interval between the single heart beats Some examples of obtained recordings are given

Fields of possible application of cardiotachygraphy seem to be numerous The instrument is inexpensive and simple to handle

Acknowledgement

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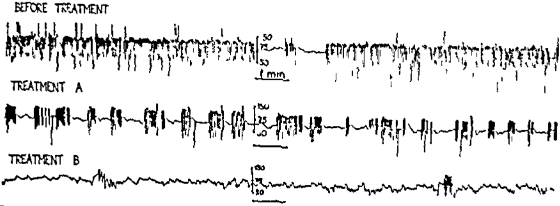


Fig 13 Arrhythmia due to abundance of ventricular extrasystoles, treated with two different agents A and B

Discussion

The heart rate has attracted much attention in clinical practice Isolated observation of the heart rate by pulse rate counting, or from ECGs, has many sources of error inherent in it They range from simple tachycardia due to patient stress to more serious problems of missing transitory changes in rhythm Devices for continuous heart rate recording have been described long ago (1,2,5) In clinical practice they have usually been unsatisfactory for a variety of technical reasons Advances in engineering electronics in the last decade have made available means of avoiding many of these problems A number of solutions have been put forward (3, 4, 7, 8, 12, 13, 14, 16, 17, 18, 19, 20) However, so far the methods described rarely seem to have received clinical application

At the beginning of this work it was considered of value to determine the total number of heart beats over 24 hours In some situations such values might be of a certain interest, as for example from patients with thyrotoxicosis cardiac failure, neurocirculatory asthenia and others This can easily be obtained by allowing the mechanical register to work while the paper recording device is disconnected, as 24 hours represent almost 30 meters of recording paper

Cardiotachygraphy as described in this report can be considered as a simple and inexpensive method of continuous long-term recording of heart rate and of the accumulated number of heart-beats Fields of possible application are numerous, ranging from physiology to psychiatry, from obstetrics to rehabilitation

Summary

The aim of this report is to describe a specially designed instrument for continuous long term recording of heart rate, called the cardiotachygraph

It works in connection with a conventional electrocardiograph thus making simultaneous ECG-recordings possible In principle, the R waves act as trigger pulses that actuate a linear sweep circuit The peak voltage is fed to a registration instrument and the obtained curve will continuously demonstrate the length of each RR-interval, and thus

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Inhibitory Effect of Nicotinic Acid on FFA Mobilization in Alloxan-diabetic Rats

I In Vitro Studies on the Fatty Acid Metabolism in Adipose Tissue after Nicotinic Acid Administration to Donor Animals[1]

By

JAN ÖSTMAN

It has recently been shown that the administration of nicotinic acid (NiAc) depresses the concentration of unesterified (free) fatty acids (FFA) in plasma of rats in alloxan diabetic state (5), a condition concomitant with an elevated concentration of plasma FFA (13) and an increased mobilization of FFA from adipose tissue *in vitro* (1 11, 12) It has also been observed that NiAc *in vitro* inhibits the net release of FFA from adipose tissue of alloxan diabetic rats (14) An inhibition of the release of FFA and glycerol from adipose tissue was demonstrated simultaneously with an increased incorporation of labelled palmitate from the medium into the neutral tissue lipids For that reason it is likely that the lowering effect of NiAc on the plasma FFA of alloxan diabetic rats is due to a decrease in the mobilization of FFA from the fat depots To ascertain this the fatty acid metabolism was studied *in vitro* on epididymal fat pads removed from alloxan diabetic rats pretreated with NiAc A number of the animals included in a study reported elsewhere (5) were used for the present investigation

Submitted for publication December 16 1964

Material and methods

Diabetic animals were prepared by subcutaneous administration of alloxan (150 mg/kg body weight) and were not used until three weeks later when the blood glucose concentration exceeded 350 mg/100 ml in non fasting state At the same time blood samples were collected from the tail vein for determination of plasma triglycerides Among 32 diabetic animals included in the previously reported study (5) twelve were selected for the *in vitro* experiments on adipose tissue The animals were so chosen that with respect of the initial concentrations of

[1] Reported in part at the Meeting of British and Swedish Biochemical Societies in Stock holm on May 15 1964

from the neck wound were taken for determinations of blood glucose, plasma FFA and plasma triglycerides. Epididymal fat pads were removed whole but in two animals no adipose tissue was found.

For that reason the fat pads were removed from two other diabetic animals included as controls in the simultaneous series of experiments dealing with the effect of NiAc on the plasma lipids. The consequence was that the initial equalization of the two groups of animals was disarranged.

After 30 minutes of preincubation at 37 C in unlabelled albumin-containing medium the paired fat pads were transferred to 4 ml of Krebs-Henseleit bicarbonate buffer containing 50 mg/ml of human albumin (Kab AB, Stockholm, Sweden) and C labelled palmitate (Radiochemical Centre, Amersham, England). The medium concentration of FFA was 1 17 μEq/ml and the specific activity of FFA approximately 85 000 c p m /μEq. After two hours of incubation samples from the medium were taken for determinations of FFA and glycerol. In the control medium lacking tissue the amount of radioactivity was also determined. The content of FFA and the amount of radioactivity recovered as neutral lipids and FFA in tissue were determined as recently described (15). Incubation methods including chemical and radioactive assays have been described elsewhere (14).

Determinations in blood and plasma

Blood glucose was determined by Marks procedure (8), plasma FFA according to Dole's technique (6) as modified by Trout et al. (10) and plasma triglycerides by the method of Carlson and Wadstrom (2) as modified by Carlson (4).

Calculations

In order to estimate the output of FFA from tissue and the uptake of FFA into tissue the formula devised by Dole was used (7) based on the changes in the concentration of FFA and the amount of radioactivity in the medium during the incubation. The difference between the initial medium and the activity recovered within tissue was used as an estimate of the final medium activity.

Statistical calculations were made as recommended by Snedecor (9).

Results

Table I shows the body weight and the concentrations of blood glucose and plasma triglycerides of the control

...olism of epididymal fat pads of alloxan-diabetic rats. Mean ± S E for 6 animals in each group

Radioactivity taken up from medium		Calculated number μEq FFA taken up from medium	
Tissue neutral lipids c p m g	Tissue FFA (c p m /g)	Neutral lipids	FFA
45 000 ± 3,200	9 900 ± 500	0 64 ± 0 05	0 14 ± 0 02
81 300 ± 6 700	12 000 ± 3 300	0 96 ± 0 08	0 14 ± 0 04
0 001	> 0 05	< 0 01	> 0 05
56 900 ± 5 200	12 100 ± 2 500	0 78 ± 0 04	0 17 ± 0 02
67 100 ± 11 000	7 700 ± 4 100	0 75 ± 0 12	0 09 ± 0 02
0 05	> 0 05	> 0 05	< 0 025

Wet weight of paired fat pads: Control group 1 274 ± 0 119 g, NiAc 0 790 ± 0 145 g ($p < 0.05$)

TABLE I Body weight, blood glucose, and plasma triglycerides in alloxan diabetic rats before and after three weeks' treatment with nicotinic acid Mean ± S E for 6 animals in each group

		Group	Body weight (g)	Blood glucose (mg/100 ml)	Plasma triglycerides (mMole/l)
Before treatment		Control	261 5 ± 6 6	420 2 ± 21 2	0 93 ± 0 10
		NiAc	227 2 ± 14 3	474 3 ± 26 7	1 75 ± 0 21
	p		> 0 05	> 0 05	< 0 01
After treatment		Control	295 7 ± 8 5	430 2 ± 30 9	1 22 ± 0 09
		NiAc	236 0 ± 16 2	423 3 ± 33 4	1 18 ± 0 19
	p		< 0 01	> 0 05	> 0 05
Individual changes during treatment		Control	+ 34 2 ± 4 4	+ 10 0 ± 4 3	+ 0 29 ± 0 20
		NiAc	+ 8 8 ± 10 7	— 51 0 ± 33 6	— 0 57 ± 0 27
	p		> 0 05	> 0 05	< 0 025[1]

[1] Calculated on logarithmic values (p < 0 025)

blood glucose and plasma triglycerides, and of the initial body weight, they consisted of two comparable groups, one receiving NiAc and the other serving as control group NiAc was given subcutaneously in a daily dosage of 150 mg/kg body weight for 17 days and in a dosage of 300 mg/kg body weight for the last four days The control animals received injections of saline, the solvent used for NiAc Food and water were given *ad libitum* during the whole period of treatment The experimental conditions are described in detail elsewhere (5)

In vitro procedure

The rats were killed by decapitation in non fasting state two hours after the last injection of NiAc and saline Blood samples

TABLE II In vitro effect of pretreatment of donor animals with nicotinic acid on fatty acid metab

		Group	Glycerol release (μMole/2 hrs)	FFA net release (μEq/2 hrs)	Tissue FFA (μEq)
Per wet weight of tissue (g)		Control	1 79 ± 0 26	2 58 ± 0 55	2 81 ± 0 46
		NiAc	1 44 ± 0 16	0 85 ± 0 22	2 47 ± 0 25
	p		> 0 05	< 0 025	> 0 05
Per paired fat pads[1]		Control	2 17 ± 0 22	3 01 ± 0 88	1 76 ± 0 31
		NiAc	1 06 ± 0 15	0 53 ± 0 07	1 00 ± 0 17
	p		< 0 005	< 0 05	> 0 05

[1] Calculated from the mean specific activity (7) of medium FFA

from the neck wound were taken for determinations of blood glucose plasma FFA and plasma triglycerides Epididymal fat pads were removed whole but in two animals no adipose tissue was found

For that reason the fat pads were removed from two other diabetic animals included as controls in the simultaneous series of experiments dealing with the effect of NiAc on the plasma lipids The consequence was that the initial equalization of the two groups of animals was disarranged

After 30 minutes of preincubation at 37 C in unlabelled albumin containing medium the paired fat pads were transferred to 4 ml of Krebs-Henseleit bicarbonate buffer containing 50 mg/ml of human albumin (Kabi AB Stockholm Sweden) and C labelled palmitate (Radiochemical Centre Amersham England) The medium concentration of FFA was 1 17 μEq/ml and the specific activity of FFA approximately 85 000 c p m /μEq After two hours of incubation samples from the medium were taken for determinations of FFA and glycerol In the control medium lacking tissue the amount of radioactivity was also determined The content of FFA and the amount of radioactivity recovered as neutral lipids and FFA in tissue were determined as recently described (15) Incubation methods including chemical and radioactive assays have been described elsewhere (14)

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Table I shows the body weight and the concentrations of blood glucose and plasma triglycerides of the control

ol sm of epididymal fat pads of alloxan diabetic rats Mean ± S E for 6 animals in each group

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Tissue neutral lipids (c p m g	Tissue FFA c p m g)	Neutral lipids	FFA
45 000 ± 3 200	9 900 ± 500	0 64 ± 0 03	0 14 ± 0 02
84 300 ± 6 700	12 000 ± 3 300	0 96 ± 0 08	0 14 ± 0 04
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67 100 11 000	7 700 ± 4 100	0 75 ± 0 1°	0 09 ± 0 02
0 05	0 05	>0 05	<0 025

Wet weight of paired fat pads Control group 1 274 ± 0 119 g NiAc 0 793 ± 0 145g (p<0 0)

TABLE I Body weight, blood glucose, and plasma triglycerides in alloxan-diabetic rats before and after three weeks treatment with nicotinic acid Mean ± S E for 6 animals in each group

		Group	Body weight (g)	Blood glucose (mg/100 ml)	Plasma triglycerides (mMole/l)
Before treatment		Control	261.5± 6.6	420.2±21.2	0.93±0.10
		NiAc	227.2±14.3	474.3±26.7	1.75±0.21
	p		> 0.05	> 0.05	<0.01
After treatment		Control	295.7± 8.5	430.2±30.9	1.22±0.09
		NiAc	236.0±16.2	423.3±33.4	1.18±0.19
	p		< 0.01	> 0.05	>0.05
Individual changes during treatment		Control	+ 34.2± 4.4	+ 10.0± 4.3	+0.29±0.[illegible]0
		NiAc	+ 8.8±10.7	— 51.0±33.6	—0.57±0.22
	p		> 0.05	> 0.05	<0.025[1]

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		Group	Glycerol release (μMole/2 hrs)	FFA net release (μEq/2 hrs)	Tissue FFA (μEq)
Per wet weight of tissue (g)		Control	1.79±0.26	2.58±0.55	2.81±0.46
		NiAc	1.44±0.16	0.85±0.22	2.47±0.25
	p		>0.05	<0.025	>0.05
Per paired fat pads[1]		Control	2.17±0.22	3.01±0.88	1.76±0.31
		NiAc	1.06±0.15	0.53±0.07	1.00±0.17
	p		<0.005	<0.05	>0.05

[1] Calculated from the mean specific activity (7) of medium FFA

from the neck wound were taken for determinations of blood glucose, plasma FFA and plasma triglycerides. Epididymal fat pads were removed whole but in two animals no adipose tissue was found.

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Tissue neutral lipids (c.p.m./g)	Tissue FFA (c.p.m./g)	Neutral lipids	FFA
43 000 ± 3,200	9 900 ± 500	0.64 ± 0.05	0.14 ± 0.02
81 300 ± 6 700	12 000 ± 3 300	0.96 ± 0.08	0.14 ± 0.04
0.001	> 0.05	< 0.01	> 0.05
56 900 ± 5,200	12 100 ± 2 300	0.78 ± 0.04	0.17 ± 0.02
67 100 ± 11 000	7 700 ± 4 100	0.75 ± 0.12	0.09 ± 0.02
> 0.05	> 0.05	> 0.05	< 0.025

Wet weight of paired fat pads: Control group 1.274 ± 0.119 g; NiAc 0.793 ± 0.145 g ($p < 0.05$)

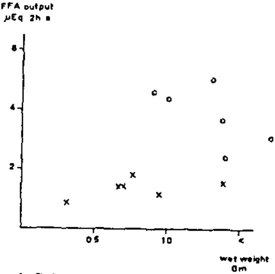


Fig 1 Relationship between FFA output and wet weight of paired fat pads of nicotinic acid pretreated (x) and untreated (o) alloxan diabetic rats ($r = 0\ 43$, $p > 0\ 6$)

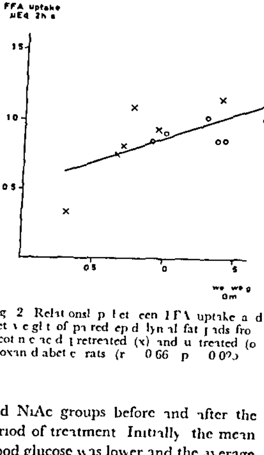


Fig 2 Relationship between FFA uptake and wet weight of paired epididymal fat pads from nicotinic acid pretreated (x) and untreated (o) alloxan diabetic rats ($r = 0\ 66$, $p < 0\ 025$)

and NiAc groups before and after the period of treatment Initially the mean blood glucose was lower and the average body weight higher in the control animals, the difference between the groups was not significant During the period of treatment the body weight increased significantly only in the control group in which it was significantly higher than in the NiAc group after the treatment ($p < 0\ 01$) At that time the blood glucose concentration did not differ significantly between the groups The effect of NiAc was not significant on blood glucose or body weight however judging from the individual changes during the period of treatment The concentration of plasma triglycerides which initially was significantly higher ($p < 0\ 01$) in the NiAc group decreased significantly ($p < 0\ 05$) during the period of treatment whereas no alteration was noted in the control group The lowering effect of NiAc on the plasma triglycerides was significant ($p < 0\ 025$) At the time of the *in vitro* experiments plasma FFA was higher in the control group $0\ 50 \pm 0\ 05$ mEq/l (mean $\pm$ S E) than in the NiAc group in which it was $0\ 35 \pm 0\ 03$ mEq/l this difference was significant ($p < 0\ 05$)

Data from the *in vitro* experiments are summarized in table II which presents the parameters expressed per paired fat pads as well as per wet weight A significantly lower net release of FFA was demonstrated from tissues of the NiAc group the difference between the groups was significant when calculated per paired fat pads ($p < 0\ 05$) as well as per wet weight ($p < 0\ 025$) Also the mean wet weight of the fat pads was higher in the control group the difference between the groups being significant ($p < 0\ 05$)

The release of glycerol was significantly lower ($p < 0.005$) from tissue of the NiAc treated animals only when expressed per paired fat pads A significantly greater amount of radioactivity ($p < 0.001$) was recovered as neutral lipids in tissue (per wet weight) of the NiAc group Also the estimated amount of medium FFA recovered as neutral tissue lipids was significantly greater in this group the difference between the groups was even now significant ($p < 0.01$) The part of total tissue activity recovered as FFA appeared to be smaller in tissue of the NiAc treated animals

In figs 1 and 2 the output of FFA and the uptake of FFA respectively have been plotted along the Y axis and the wet weight of the paired fat pads along the X axis No relationship was demonstrable between the output of FFA and the wet weight the correlation coefficient (r) was for the whole material 0 43 ($p > 0.6$) It is also seen that the output of FFA was lower from all the tissues of the NiAc group In fig 2 it will be seen that the uptake of FFA was related to the wet weight ($r = 0.66$ $p < 0.025$) The ratio between the output of FFA and the uptake of FFA was significantly higher for tissues of the controls (4 25 ± 0 50 mean – S E) than for tissues of the NiAc group (1 74 ± 0 19) the difference was significant ($p < 0.001$) From fig 3 it appears that the output of FFA and the release of glycerol were well related to each other the correlation coefficient was for the whole material 0 93 p 0 001) It will be seen that the release of glycerol like the output

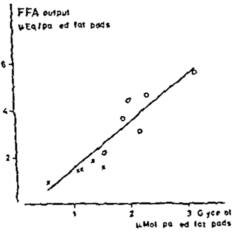


Fig 3 Relationship between FFA output and glycerol release from epididymal fat pads of nicotinic acid pretreated (x) and untreated (o) alloxan-diabetic rats ($r = 0.93$ $p < 0.001$)

of FFA was lower from all the tissues of the NiAc group and that the ratio between FFA output and glycerol release was equal for the two groups

Discussion

The present study shows that the net release of FFA *in vitro* from adipose tissue of alloxan diabetic rats were reduced by pretreatment of the donor animals with NiAc From the data it cannot be elucidated whether the changes in the fatty acid metabolism *in vitro* were related to the last injection of NiAc *per se* or reflected a more permanent alteration in the metabolic state of adipose tissue Yet the decrease in the plasma triglycerides might indicate that the mobilization of FFA from the fat depots was lowered during a great part of the period of treatment In the present series of experiments neither a decrease in the blood glucose concentration nor an increase in the body weight

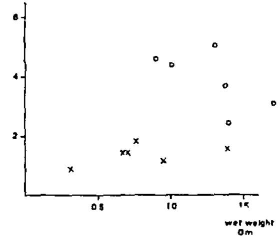


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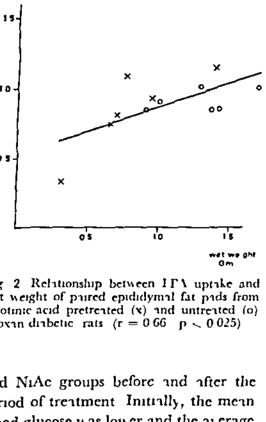


Fig 2 Relationship between FFA uptake and wet weight of paired epididymal fat pads from nicotinic acid pretreated (x) and untreated (o) alloxan diabetic rats ($r = 0.66$ $p < 0.025$)

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Data from the *in vitro* experiments are summarized in table II, which presents the parameters expressed per paired fat pads as well as per wet weight A significantly lower net release of FFA was demonstrated from tissues of the NiAc group, the difference between the groups was significant when calculated per paired fat pads ($p < 0.05$) as well as per wet weight ($p < 0.025$) Also the mean wet weight of the fat pads was higher in the control group, the difference between the groups being significant ($p < 0.05$)

The output of FFA and the release of glycerol were lower from tissue of animals pretreated with nicotinic acid An increased incorporation of labelled palmitate into the neutral tissue lipids was also observed When the data are taken together, it seems reasonable to regard the primary action of nicotinic acid in adipose tissue of alloxan diabetic rats a an inhibition of the triglyceride breakdown

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were evoked by the treatment, which is in contrast with the previous studies (5). Since the diabetic state was initially more severe in the NiAc group of the present study, one cannot exclude a beneficial effect of NiAc on alloxan-diabetic states, with respect to blood glucose and body weight. However, the treatment with NiAc decreased the mobilization of FFA from the fat depots in this series of experiments, without blood glucose and body weight being altered significantly during the same period. A diminished net release of FFA from adipose tissue *in vitro* would be explained by a decreased release of FFA from and/or an increased uptake of FFA into adipose tissue. In this study it was shown that the output of FFA as well as the release of glycerol were lower from all the tissues of the NiAc-treated animals. Since the output of FFA and the release of glycerol were well related to each other, the findings suggest that the decreased net release of FFA can be explained by a decreased triglyceride breakdown. Whereas no relationship was demonstrated between the output of FFA and the wet weight of the paired fat pads, a positive relationship was observed between the uptake of FFA and the wet weight of the fat portions. For that reason the uptake of FFA was expressed per wet weight in comparing this parameter between tissues of the two groups. A significantly greater uptake of FFA and a concomitantly greater incorporation of labelled palmitate into the neutral tissue lipids were then demonstrated in the NiAc group. However, bearing in mind the absolute values of the parameters (figs. 1—3), the increase in FFA uptake appears to be far less pronounced than the decrease in FFA output and glycerol release. It should be emphasized that an increased incorporation of labelled palmitate into the neutral lipid fraction in adipose tissue does not imply an increase in the esterification of FFA. Similar findings would be obtained also under other conditions when there is an increase in the specific activity of FFA in the immediate precursor pool for the triglyceride synthesis. Thus an inhibition of the triglyceride breakdown would compress the FFA pool and hence increase the specific activity of FFA. The data obtained would be compatible with the hypothesis that NiAc inhibits the triglyceride breakdown in adipose tissue of alloxan diabetic rats and that the increased incorporation of palmitate into the neutral tissue lipids is a secondary phenomenon.

Summary

The fatty acid metabolism was studied in isolated epididymal fat pads from alloxan diabetic rats treated for three weeks with nicotinic acid and compared with that of tissue from untreated alloxan diabetic animals. The net release of FFA was lower from adipose tissue of animals pretreated with nicotinic acid. This finding is on a par with the view that nicotinic acid depresses the plasma FFA in alloxan diabetic rats by reducing the mobilization of FFA from the fat depots. In addition the plasma triglycerides were depressed by the treatment, whereas neither the blood glucose concentration nor the body weight were influenced.

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Inhibitory Effect of Nicotinic Acid on FFA Mobilization in Alloxan-diabetic Rats

II A Comparison of the Effect of Nicotinic Acid and Salicylate on the Fatty Acid Metabolism and Glucose Uptake by Adipose Tissue in Vitro

By

JAN ÖSTMAN

Nicotinic acid (NiAc) (5, 6, 7), and salicylate (Sal) (4 8) are both agents which in acute experiments rapidly depress the concentration of free fatty acids (FFA) in plasma of normal and diabetic man apparently by inhibiting the mobilization of FFA from the fat depots (4 7) Under these conditions NiAc does not affect the concentration of blood glucose (9 16 19 27) whereas Sal produces a fall of blood glucose which is moderate and slow in onset (4 8 11 15 17) NiAc (1 6 14 31) and Sal (4 8) have been shown also to inhibit the net release of glycerol as well as FFA from rat adipose tissue *in vitro* observed in fasting conditions and in conditions with activated lipolysis in adipose tissue This suggests that the effect of both agents on the fatty acid metabolism is unrelated to an increased uptake of glucose into this tissue

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Previous *in vitro* experiments (31) have shown that NiAc also enhances the incorporation of labelled medium FFA into neutral lipids of the adipose tissue obtained from alloxan diabetic rats The present investigations were made in order to find out whether this effect would also be demonstrated with Sal, in other words whether another agent than NiAc, with apparent inhibitory action on the triglyceride breakdown, would also increase the incorporation of labelled FFA into the tissue triglycerides

Material and methods

Epididymal adipose tissue was obtained from diabetic rats three weeks after the injection of alloxan At the time of removal of the fat pads from the animals in non fasting state the glucose concentration in venous blood ranged from 300 to 400 mg/100 ml After 30 minutes of preincubation in an albumin buffer solution the fat pads were transferred

cerol uptake of C[14] labelled palmitate and uptake of glucose by epididymal adipose tissue of alloxan pads obtained from four or five animals and incubated in media of different types

Radioactivity incorporated (c.p m./g)		Calculated number μEq FFA taken up from medium/g tissue	Tissue FFA (μEq/g)	Glucose uptake (mg/g/2 hrs)
Neutral lipids	FFA	Neutral lipids		
177 800	32 400	0 50	6 33	1 95
163 600	26,200	0 62	4 24	2 31
— 14 200±8 100	— 6,200±10 500	+0 12±0 18	+2 09±1 67	+0 36±0 04
>0 05	>0 05	>0 05	>0 05	<0 001
408 800	24 600	1 68	1 00	1 12
477,200	15,200	1 73	0 97	0 95
+ 68 400±25 400	— 9 400±8 000	+0 04±0 46	+0 03±0 10	—0 16±0 28
>0 05	>0 05	>0 05	>0 05	>0 05
27 000	10 900	0 30	4 91	—
31 700	16 400	0 34	4 88	—
+ 4 700± 1 900	+ 5 500± 1 500	+0 04±0 02	—0 03±0 10	—
0 1>p>0 05	<0 025	>0 05	>0 05	—

(NiAc) 10 [-4]M [a] Individual differences of contralateral fat pads [b] Calculated from the mean

Results

From table I it will be seen that both Sal (A) and NiAc (B) inhibit the net release of FFA (Sal $p < 0.001$, NiAc $p < 0.05$) and glycerol (Sal $p < 0.025$ NiAc $p < 0.05$) from adipose tissue of alloxan diabetic rats No significant increase in the amount of radioactivity recovered as neutral lipids was observed *in tissue incubated in the* medium containing NiAc and no effect was demonstrated in the presence of Sal In view of the changes in the specific activity of medium FFA *during* the incubation the amount of medium FFA recovered as neutral tissue lipids was calculated from the mean specific activity of medium FFA as described by Dole (13) Even when so calculated, the incorporation *of* medium FFA into neutral tissue lipids was *not* significantly increased by NiAc and unaffected by Sal No agent altered the concentration of tissue FFA or the amount of radioactivity *recovered* as FFA in tissue The uptake of glucose was *significantly increased* ($p < 0.001$) by Sal but unaffected by NiAc

In another series of *experiments* (table I C) the effect of Sal was studied in medium without added glucose Also in these experiments Sal was shown to inhibit significantly the net release of FFA ($p < 0.05$) and glycerol ($p < 0.05$) from adipose tissue A slightly increased

TABLE I In vitro effect of sodium salicylate and nicotinic acid on net release of FFA, release of gl[illegible] diabetic rats Mean values are given in each series of experiments for the contralateral fat [illegible]

	Added to medium[1]	Glycerol release (μMole/g/2 hrs)	FFA net release (μEq/g/2 hrs)
Exp A	Glucose	4 47	1 67
n=4	SalAc+Glucose	2 34	0 12
	Mean difference ± S E[2]	—2 13±0 53	—1 55±0 16
	p	<0 025	<0 001
Exp B	Glucose	2 51	0 89
n=5	NiAc+Glucose	0 95	0 08
	Mean difference ± S E[2]	—1 55±0 53	—0 81±0 29
	p	<0 05	<0 05
Exp C	O	2 19	3 95
n=4	SalAc	1 89	3 20
	Mean difference ± S E[2]	—0 30±0 09	—0 75±0 22
	p	<0 05	<0 05

[1] At final concentrations glucose 2 mg/ml, sodium salicylate (Sal) 5 10 $^{-3}$M and nicotinic acid [illegible]
specific activity of medium FFA estimated according to the formula devised by Dole (13)

into a medium consisting of 5 ml (unless otherwise specified) of Krebs Henseleit bicarbonate buffer, containing 50 mg/ml of human albumin (Kabi AB Stockholm, Sweden) and C[14]-labelled palmitate (Radio chemical Centre, Amersham England) and, in some experiments 2 mg/ml of glucose In various series of experiments nicotinic acid and sodium salicylate were studied added to the medium at a final concentration of 10 $^{-4}$M and 5 10 $^{-3}$M respectively One fat pad was incubated in the presence of the agent tested All incubations were carried out for two hours in a 37 C water bath with air as gas phase

At the end of the incubation samples of the medium were taken for determinations of FFA (12 26), glycerol (30) and glucose (18) From the control medium included in each series of experiments and lacking tissue the amount of radioactivity was also determined The content of FFA and the radioactivity recovered as neutral lipids and FFA within tissue were determined as described in detail elsewhere (32) Since sodium salicylate interferes with the determination of FFA (2, 10) by the afore mentioned methods (12 26) a modification of these procedures was used in the experiments where salicylate was included From the upper heptane layer obtained by Dole s extractant, salicylate was washed out by two successive extractions with 0 05 % H_2SO_4 The incubation method, including chemical and radioactive assays has been described in detail elsewhere (31, 32)

Statistical calculations were made as recommended by Snedecor (23) The uptake of medium FFA into neutral lipid and FFA fractions of the tissue were calculated from the radioactivity recovered and the mean specific activity (13) of the medium FFA

NiAc produced no significant increase in the incorporation of activity into neutral lipids in glucose free medium and had no effect on the concentration of tissue FFA This is most likely explained by the fact that the esterification was maximally increased by the supply of glucose in the medium in the present study The further increase in the incorporation of activity into neutral lipids could not be attributed to any stimulation by NiAc on the uptake of glucose On the other hand, Sal produced an increased uptake of glucose which is in accordance with previous studies showing that Sal increases the production of $C^{14}O_2$ from uniformly labelled glucose in isolated adipose tissue of normal rat (22) From the experiments it has not been made clear why the increase in the uptake of glucose was unaccompanied by an increase in the esterification of FFA

Since in glucose free medium NiAc unlike Sal increases the incorporation of radiopalmitate into the neutral tissue lipids the agents seem to inhibit the release of FFA by different mechanisms For reasons recently discussed (25 29) an increased incorporation of activity into the neutral tissue lipids observed in the NiAc experiments, does not simply indicate an increased esterification of FFA In this study it was shown that an increased incorporation of activity into the neutral tissue lipids occurred in the presence of NiAc even under conditions when the specific activity of medium FFA was kept constant Thus the increased incorporation of activity into the neutral lipids seems to be independent of an increased specific activity of the medium FFA, but is related to the effects of NiAc on fatty acid metabolism in tissue Some support for the concept (1, 5, 6, 14) that the effect of NiAc is primarily an inhibition of the lipolysis and not an increased esterification, has recently been given from studies on isolated adipose tissue after NiAc treatment of the donor animals (33) In that study the increase in the incorporation of labelled palmitate into the neutral lipids was far less than the decrease in the output of FFA and release of glycerol from adipose tissue

From the present experiments one might assume that the effect of salicylate is not an increased esterification of FFA, a process regulated by factors such as alpha glycerophosphate and ATP (20, 24) but is a direct effect on one of the lipolytic systems in adipose tissue (21, 28) If so, only one of the agents with inhibitory effect on the lipolytic systems in adipose tissue i e NiAc, is concomitant with an increased incorporation of labelled palmitate into the neutral lipids Of interest is that 2 4 dinitrophenol which like Sal inhibits the oxidative phosphorylation does not decrease the net release of FFA from rat adipose tissue when added *in vitro* at a concentration of 5 10 ⁴M to the incubation medium (3)

From the present investigation one might suggest that the unequal effects of NiAc and Sal on the fatty acid as well as the glucose metabolism in adipose tissue *in vitro* might be exploited as tools for studies on the metabolic pathways in this tissue

TABLE II Effect of nicotinic acid on incorporation of C^{14} labelled palmitate into neutral lipids of epididymal fat tissue from alloxan diabetic rats when concentration and radioactivity of FFA were kept constant during incubation in medium of large volume. Nicotinic acid (NiAc) was added to the medium at a final concentration of 10^{-4} M. Basal incubation medium: 50 ml of Krebs Henseleit bicarbonate buffer containing 10 mg/ml of human albumin (providing 0.18 μEq/ml medium), and palmitic acid 1-C^{14} in an amount corresponding to 122,000 counts/min/ml

Rat no	Added to medium	Medium FFA: Final conc (μEq/ml)	Medium FFA: Final radioactivity (c p m /ml)	Medium FFA: Net release (μE/ml/g/2 hrs)	Tissue FFA: Radioactivity (c p m /g)	Tissue FFA: Specific activity (c p m /μEq)	Tissue neutral lipids: Radioactivity (c p m /g)	Tissue neutral lipids: % total activity
1	0	0.21	111,500	0.17	105,600	517,600	33,700	24
	NiAc	0.18	111,450	0.05	98,900	231,600	209,000	68
2	0	0.18	110,960	0.10	80,300	275,800	37,700	32
	NiAc	0.18	110,840	0.02	43,600	237,000	50,400	54
3	0	0.20	111,200	0.06	76,600	254,600	50,800	40
	NiAc	0.18	110,940	0.06	47,400	141,000	56,000	60

($0.1 > p > 0.05$) incorporation of labelled palmitate was noted into the neutral tissue lipids. However, no effect was demonstrated when estimating the uptake of FFA into this fraction by use of the mean specific activity of medium FFA. Neither the concentration of FFA nor the amount of activity recovered as FFA in tissue was influenced by Sal.

Table II shows the effect of NiAc when adipose tissue was incubated in a medium of large volume (50 ml). While some decrease in the net release of FFA from adipose tissue was observed, neither the amount of medium FFA nor the amount of medium activity changed appreciably during the incubation. The percentage of total tissue activity recovered as neutral lipids was significantly greater in NiAc containing medium, whereas no consistent effect on the total uptake of activity appeared. The specific activity of FFA tended to be lower in the tissue incubated in the presence of NiAc.

Discussion

In these studies, Sal, like NiAc, was shown to inhibit the net release of glycerol and FFA from adipose tissue of alloxan diabetic rats whether or not the tissues were incubated in medium containing glucose. In neither type of medium was the incorporation of activity into neutral tissue lipids increased or the tissue concentration of FFA depressed, findings which have previously been observed in the presence of NiAc (31). In the present investigation

NiAc produced no significant increase in the incorporation of activity into neutral lipids in glucose free medium and had no effect on the concentration of tissue FFA This is most likely explained by the fact that the esterification was maximally increased by the supply of glucose in the medium in the present study The further increase in the incorporation of activity into neutral lipids could not be attributed to any stimulation by NiAc on the uptake of glucose On the other hand, Sal produced an increased uptake of glucose, which is in accordance with previous studies showing that Sal increases the production of $C^{14}O_2$ from uniformly labelled glucose in isolated adipose tissue of normal rat (22) From the experiments it has not been made clear why the increase in the uptake of glucose was unaccompanied by an increase in the esterification of FFA

Since in glucose free medium NiAc unlike Sal, increases the *incorporation* of radiopalmitate into the neutral tissue lipids the agents seem to inhibit the release of FFA by different mechanisms For reasons recently discussed (25, 29) an increased incorporation of activity into the neutral tissue lipids, observed in the NiAc experiments does not simply indicate an increased esterification of FFA In this study it was shown that an increased incorporation of activity into the neutral tissue lipids occurred in the presence of NiAc even under conditions when the specific activity of medium FFA was kept constant Thus the increased incorporation of activity into the neutral lipids seems to be independent of an increased specific activity of the medium FFA, but is related to the effects of NiAc on fatty acid metabolism in tissue Some support for the concept (1, 5, 6, 14) that the effect of NiAc is primarily an inhibition of the lipolysis, and not an increased esterification, has recently been given from studies on isolated adipose tissue after NiAc treatment of the donor animals (33) In that study the increase in the incorporation of labelled palmitate into the neutral lipids was far less than the decrease in the output of FFA and release of glycerol from adipose tissue

From the present experiments one might assume that the effect of salicylate is not an increased esterification of FFA, a process regulated by factors such as alpha glycerophosphate and ATP (20, 24), but is a direct effect on one of the lipolytic systems in adipose tissue (21 28) If so only one of the agents with inhibitory effect on the lipolytic systems in adipose tissue, i e NiAc is concomitant with an increased incorporation of labelled palmitate into the neutral lipids Of interest is that 2 4 dinitrophenol, which like Sal inhibits the oxidative phosphorylation does not decrease the net release of FFA from rat adipose tissue when added *in vitro* at a concentration of 5 10^{-4}M to the incubation medium (3)

From the present investigation one might suggest that the unequal effects of NiAc and Sal on the fatty acid as well as the glucose metabolism in adipose tissue *in vitro* might be exploited as tools for studies on the metabolic pathways in this tissue

Summary

Sodium salicylate, like nicotinic acid, was found to inhibit *in vitro* the net release of FFA and glycerol from epididymal adipose tissue of alloxan-diabetic rats The uptake of FFA and the esterification of FFA were unaffected by salicylate when measured by mean of labelled palmitate added to the incubation medium Apparently the action of salicylate on the fatty acid metabolism differs from that of nicotinic acid, since in the presence of this agent there is an increase in the amount of radiopalmitate esterified to neutral lipids in tissue

An increased uptake of glucose was demonstrated by salicylate, while no effect was seen in the presence of nicotinic acid

The action of the agents on the fatty acid metabolism in adipose tissue is discussed on the basis of the results

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Inhibition of the Mobilization of Free Fatty Acids from Adipose Tissue in Diabetes

I Effect of Nicotinic Acid on the Alloxan diabetic State in Rats

By

Lars A Carlson and Jan Östman

During incubation of skeletal muscle (16) and perfusion of the rat heart (16, 22, 24), the uptake of glucose from the medium is related to the concentration of FFA When the FFA level is increased in such *in vitro* systems the glucose uptake is depressed Furthermore it is known that the concentration of free fatty acid (FFA) in plasma is elevated in the diabetic state in man (2, 18) and in animals (27 31) These findings have led to the hypothesis that one factor involved in the pathogenesis of the hyperglycemia of diabetes mellitus may be an inhibition of the uptake of glucose in muscles caused by the elevated level of FFA in plasma (23)

It is also known that the increased concentration of FFA in plasma in the diabetic state can at least partly be ascribed to an increased rate of mobilization of FFA from adipose tissue Thus, when adipose tissue from alloxan-diabetic rats (5, 17 29 30 31) and from diabetic patients (12 33) is incubated *in vitro* a greater release of FFA and glycerol occurs than when adipose tissue from healthy controls is incubated The present series of studies have been designed to test the hypothesis relating hyperglycemia to increased FFA levels in plasma by inhibiting the mobilization of FFA from adipose tissue in diabetes and studying the effects of this inhibition on carbohydrate and lipid metabolism

Nicotinic acid inhibits the mobilization of FFA from normal (10) and alloxan diabetic (32) adipose tissue and decreases the concentration of FFA in plasma (8 11 13 21) In this study we have treated alloxan diabetic rats with nicotinic acid in order to inhibit the mobilization of FFA The effect of this treatment on the diabetic state will be reported

Material and methods

Adult male rats weighing 200—220 g and all of the same age were injected subcutaneously with alloxan monohydrate (15 mg/

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TABLE I The effect of nicotinic acid on the concentration of FFA in plasma of alloxan diabetic rats killed at the end of the study 2 hours after injection of saline (D) or nicotinic acid (DN) Food and water given ad libitum Mean value ± S E of mean

Group	Plasma FFA (mEq/l)
D n=15	0 67±0 06
DN n=16	0 43±0 04
p	<0 001

D=Alloxan-diabetic rats, DN=Alloxan diabetic rats treated with nicotinic acid

100 g body weight) after fasting for 24 hours Only animals which after one month *(period I) had blood glucose values exceeding* 350 mg/100 ml in a nonfasting state were included in the study Thirty two alloxan-diabetic rats thus obtained were divided into two groups, stratified according to the concentrations of blood glucose and plasma triglycerides at that time During the study one of the control animals died, presumably due to the diabetic state

For the following 14 days (period II) 15 mg/100 g body weight of nicotinic acid per day was given to one group (DN), while the other (D), serving as the control, received saline During the next week (period III) the same dose of nicotinic acid was given for three days, whereafter 30 mg/100 g body weight was administered during the remaining four days

Standard rat food (Rat food 210, Anticimex, Stockholm, Sweden) and water was given *ad libitum* Nicotinic acid dissolved in saline (15 mg/ml) was injected subcutaneously at 7 a m and 7 p m

At the end of each period, and two hours after the preceding injection in the morning blood samples were removed from the tail vein for determinations of blood glucose, plasma triglycerides and cholesterol At the same time the body weight was determined

After period III the animals were killed by *decapitation* two hours after the last injection in the morning Blood samples obtained from the wound were removed into heparinized tubes for determinations of plasma FFA in addition to blood glucose plasma triglycerides and plasma cholesterol The liver was quickly excised and immediately deep frozen for later lipid analysis The epididymal fat pads were taken from some animals for studies on the fatty acid metabolism *in vitro*, which are reported separately (34)

Blood glucose was determined according to Marks (19) The Dole (15) procedure as modified by Trout et al (28) was used for plasma FFA Triglycerides were determined according to Carlson and Wadstrom (6) as modified by Carlson (9) Cholesterol analyses were performed as described by Sperry and Webb (26) The liver content of triglycerides, cholesterol and phospholipids was determined as described previously (9) Statistical calculations were done according to Snedecor (25)

Results

At the end of the study and two hours after the last injection the concentration of FFA in plasma was significantly lower in the group treated with nicotinic acid (table I)

The mean body weight of the rats and the concentration of blood sugar, plasma triglycerides and cholesterol in the treated and in the nontreated group of alloxan diabetic rats during the study are given in table II There was no significant difference between the two groups in the pretreatment levels of blood sugar, plasma triglycerides and cholesterol The initial body weight was, however, significantly higher in the group which received saline only As described, the concentration of blood

TABLE II The effect of nicotinic acid on body weight blood sugar plasma triglycerides and cholesterol in alloxan-diabetic rats Blood was withdrawn 2 hours after the injection of saline or nicotinic acid Food and water given ad libitum. D = alloxan-diabetic controls DN = nicotinic acid treated alloxan-diabetic animals Mean value ± S E of mean

Periods[1]		I	II	III	(II—I)	(III—I)
Body weight (g)	D	246.1 ± 11.7	252.4 ± 14.2	262.1 ± 15.4	6.3 ± 7.9	15.9 ± 8.2
	p_1				>0.05	>0.05
	DN	206.8 ± 9.2	221.6 ± 16.0	227.1 ± 10.0	14.8 ± 6.3	20.4 ± 7.0
	p_1				<0.05	<0.05
	p_2	<0.05	>0.05	>0.05	>0.05	>0.05
Blood sugar (mg/100ml)	D	475.1 ± 19.5	489.3 ± 18.9	455.8 ± 18.3	14.1 ± 22.6	−19.3 ± 26.1
	p_1				>0.05	>0.05
	DN	485.0 ± 18.7	495 ± 25.2	415.0 ± 22.0	10.9 ± 16.6	−70.0 ± 27.3
	p_1				>0.05	<0.05
	p_2	>0.05	>0.05	>0.05	>0.05	>0.05
Plasma triglycerides (mMole/l)	D	1.24 ± 0.18	2.64 ± 0.56	1.87 ± 0.31	1.40 ± 0.44	0.63 ± 0.26
	p_1				<0.01	<0.05
	DN	1.51 ± 0.18	3.56 ± 0.54	1.42 ± 0.12	2.03 ± 0.55	−0.09 ± 0.18
	p_1				<0.01	>0.05
	p_2	>0.05	>0.05	>0.05	>0.05	<0.05
Plasma cholesterol (mg/100 ml)	D	138.9 ± 4.4	120.8 ± 4.0	109.3 ± 5.7	−18.1 ± 6.1	−29.6 ± 5.6
	p_1				<0.05	<0.001
	DN	137.8 ± 7.9	106.1 ± 6.2	81.5 ± 5.4	−31.7 ± 8.6	−56.3 ± 6.6
	p_1				<0.01	<0.001
	p_2	>0.05	>0.05	<0.01	>0.05	<0.01

[1] I = End of the period (one month after the first injection of alloxan) II = End of the period (23 days after I) III End of the period (7 days after II)

p_1 Significance of change within each group p_2 significance of difference between D and DN

sugar and plasma triglycerides were used in the stratification of the rats into the two groups

The weight increased slightly in both groups during the study This increase was however only statistically significant for the nicotinic acid treated group The blood sugar showed no significant changes in either group at the end of period II but had decreased significantly in the group given nicotinic acid at the end of period III In both groups the plasma triglycerides had increased significantly at the end of period II, there being no difference between the groups At the end of period III the plasma triglycerides were still significantly above the pretreatment level in the group receiving saline while no such elevation was present in the treated group This difference between the groups is statistically significant

TABLE III The effect of nicotinic acid on the concentration of triglycerides, cholesterol and phospholipids in the liver of alloxan-diabetic rats at the time of decapitation Mean value ± S E of mean

Group	Liver		
	Triglycerides (µMole/g)	Cholesterol (mg/g)	Phospholipids (mg/g)
D	8 26±0 45	5 09±0 12	31 6±0 2
DN	8 14±0 96	5 06±0 11	30 1±0 7
P	>0 05	>0 05	>0 05

Symbols as in table I

In adult (7) as well as in newborn man (4) the plasma triglycerides are skewly distributed, which makes the validity of normal statistical formulas doubtful This could be circumvented by conversion of the data to the corresponding logarithms, which were approximately normally distributed (4, 7) As we do not have enough data on plasma triglycerides in rats to study the distribution, we have preferred to make our calculations directly on the unlogarithmized values However, when the calculations were made on values converted to logarithms either the same or a higher statistical significance was obtained in every instance where $P < 0.05$ in table II Furthermore in no instance where $P > 0.05$ did the calculations on the converted values indicate increase in the probability

The plasma cholesterol levels had decreased in the two groups at both periods This decrease was significantly greater at the end of period III for the group treated with nicotinic acid

Table III shows that there was no difference in the concentrations of triglycerides, cholesterol and phospholipids in the liver of the two groups at the end of the study

Discussion

The data have shown that, as expected, the treatment with nicotinic acid depressed the level of FFA in the plasma of the alloxan-diabetic rats This effect was present two hours after the injection but the complete duration is not known Furthermore, when adipose tissue from the rats used in this study was incubated *in vitro*, the release of FFA was significantly lower from the nicotinic acid treated group (34) This strongly suggests that the low plasma levels of FFA were due to an inhibition of the mobilization of FFA, which is in accordance with the known effects of nicotinic acid on the metabolism of FFA (8, 10, 32)

The effect obtained with nicotinic acid on the plasma triglycerides and cholesterol was also expected Nicotinic acid has been known to depress the cholesterol level in man since 1955 (1) It has been suggested that this effect is due to the inhibition of the mobilization of FFA from adipose tissue (8, 10) Furthermore, it has also been suggested that the increased blood lipid levels in diabetes are related to an increased mobilization of FFA (2, 3, 12, 17, 33) The relationship between the rate of mobilization of FFA and the level of the plasma lipoproteins has been discussed in detail elsewhere (cf 14) Available data suggest that there is generally direct proportionality between the amount of FFA mobilized and the amount of FFA taken up by the liver

The concentration of liver triglycerides may in turn vary directly with the uptake of FFA Finally, the hepatic pools of triglycerides may control the rate of synthesis of the plasma tri glycerides The effect of nicotinic acid on the concentration of cholesterol and triglycerides in the plasma of the diabetic rats might thus well be ascribed to the inhibition of the mobilization of FFA But one should then expect also a change in the concentration of tri glycerides in the liver This was evidently not the case 2 hours after the last injection in this study (table III) It is difficult to discuss this finding in relation to the hypothesis outlined above, as no time studies on liver and plasma lipids were made Further studies are needed to clarify these findings

There was a slight, but significant, improvement of the diabetic state of the rats treated with nicotinic acid as revealed by increase in body weight and decrease in blood sugar This is compatible with but does not establish, the hypothesis that increased levels of FFA may to some extent cause the hyperglycemia of diabetes For several reasons, however more studies should be carried out along this line The observed effect was slight and we do not know for how long a time during the 24 hour period the mobilization of FFA was actually depressed by nicotinic acid Furthermore nicotinic acid may well influence the diabetic state by a number of other mechanisms Of interest are the previous findings (20) that when given orally as a single dose of 40 mg/100 g body weight nicotinic acid depresses the blood glucose level in normal rats but not in alloxan diabetic rats This may support the concept that the decrease in blood glucose observed in the present study was not due to a direct hypoglycemic action of nicotinic acid As the initial body weight of the nicotinic acid treated rats was lower than that of the control animals this suggests that the former had a more severe diabetic state There was no relation ship, however, between the initial body weight and the changes in blood glucose or body weight in either group Ap parently the treatment of nicotinic acid inhibited significantly the mobilization of endogenous FFA without substantial improvement of two other parameters of the diabetic state body weight and blood glucose level

Summary

The effect of nicotinic acid treatment for three weeks was studied in alloxan diabetic rats to determine whether an inhibited mobilization of FFA from adipose tissue might improve the diabetic state In comparison with the alloxan diabetic control animals, the nicotinic acid treated rats showed decreased concentrations of FFA, triglycerides and cholesterol in plasma Within the nicotinic acid treated group there was a sig nificant increase in body weight and decrease in blood glucose during the study not seen in the controls, however the same parameters did not differ sig nificantly between the groups, nor was there significant difference in liver lipid content between the treated and un treated animals

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Studies in Vitro on Fatty Acid Metabolism of Human Subcutaneous Adipose Tissue in Diabetes Mellitus[1]

By

JAN ÖSTMAN

The elevated level of FFA (free, unesterified fatty acids) is one of the features of the disturbed lipid metabolism in uncontrolled diabetes mellitus (2, 25) and in experimentally induced insulin deficiency (39) It is generally assumed that this increase in plasma is due mainly to an augmented mobilization of FFA from the fat depots (16 24) even if direct proof of this by experiments *in vivo* is still lacking Some support for this concept is that the net release of FFA *in vitro* from adipose tissue of alloxan diabetic rats (3 42 43) and human diabetics (10) has been shown to be greater than that from tissue of non-diabetic controls The present investigations were undertaken in order to find out which of the possible mechanisms an increased breakdown of tissue triglycerides or a decrease in the synthesis, i e the esterification of FFA was responsible for the elevated mobilization of FFA from adipose tissue in human diabetes mellitus An additional purpose was to find out whether the disturbance of the FFA metabolism in adipose tissue was related to a decrease in the uptake of glucose into this tissue or not In a preliminary report on this subject (10) some aspects of the fatty acid metabolism in adipose tissue were studied in a heterogenous group of untreated and treated diabetic subjects of various types The present study was designed to compare the fatty acid metabolism of adipose tissue in greater detail in two distinctly different types of diabetes mellitus i e the insulin dependent, juvenile type and the stable, adult" type characterized by overweight and sensitivity to sulphonylurea

For that reason a method (45) has been worked out for simultaneous studies of lipolysis esterification of FFA and uptake of glucose in human adipose tissue removable by a surgical biopsy procedure from subcutaneous tissue under local anaesthesia The tissue

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[1] Presented in part at the Fifth Congress of the International Diabetes Federation in Toronto in July 1964

TABLE 1 Clinical data of the diabetic and non-diabetic subjects Mean ± S E

Group	No of subjects	Age (years)	Weight[1] (% ideal)	Skinfold thickness (mm)	Arterial blood glucose[2] (mg/100 ml)	I v glucose tolerance (k)
JN	9	21 6±2 0	93 9±2 5	8 6±0 5	87 9± 2 5	1 61±0 28
JD	9	22 2±1 6	94 8±3 4	8 7±0 5	302 7±33 0	—
AN	8	55 3±2 6	121 4±9 2	21 1±0 9	89 1± 4 2	1 43±0 11
AD	10	57 7±3 7	113 1±4 1	18 3±0 8	224 5±11 4	—

[1] According to the tables of Metropolitan Life Insurance
[2] Fasting values at the time of biopsy

In all the control subjects there was 1 no family history of diabetes mellitus, and 2 normal intravenous glucose tolerance (20) with k value exceeding 1 20 in the young and lean subjects and 1 05 in the old and obese No pathological changes were observed in the following laboratory tests urine protein urinary sediment, serum creatinine alkaline phosphatase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase serum bilirubin, thymol turbidity protein bound iodine (or, in one case normal basal metabolic rate) In no subject was there evidence of a hepatic disorder appearing from the paper electrophoresis of plasma proteins The weight to-height relationship was compared with the standard values according to the tables computed by the Metropolitan Life Insurance Company (31) An approximation of body fat was made from the thickness of subcutaneous adipose tissue (skinfold thickness) measured by the method of Keys and Brozek (23) as previously used by other authors (18 26) Skinfolds were measured at three points on the right side of the body 1 on the back just caudal to the scapula 2 at the axillary border of the pectoralis major muscle at the level of the mammilla, and 3 on the abdomen in the midclavicular line at umbilical level In each instance three determinations were made and the mean of all determinations was taken as the skinfold thickness of each subject.

Table I shows the age weight to-height relationship in percentage of the mean "ideal subcutaneous thickness, and fasting arterial blood glucose in each of the clinical groups In addition the k value of the glucose tolerance is given for the two control groups

Biopsy and incubation methods

Biopsy specimens of subcutaneous adipose tissue were removed surgically from the upper lateral area of the thigh under local anaesthesia after the subject had been fasting overnight During the biopsy procedure samples of arterial blood were collected in syringes containing EDTA Na_2 thus providing plasma used as an incubation medium At the same time blood samples were removed for determinations of FFA glycerol triglycerides cholesterol and phospholipids in plasma

Duplicate incubations of tissue samples weighing 100 to 300 mg each were performed in Krebs Henseleit bicarbonate buffer containing 50 mg of human albumin and 1 mg of glucose per ml (KHA medium) and in EDTA plasma Human albumin from the same batch (Tv Rdo 17) was used for the incubations included in the present and the previously reported study (45) Palmitic 1 C^{14} was added into each medium in an amount corresponding to approximately 65 000 counts per minute per ml The concentration of FFA in the KHA medium was about 0 50 μEq/ml medium

sections were incubated in two types of media, albumin buffer of identical composition for the whole series of experiments and plasma from the subjects studied In some investigations the effect of increasing concentrations of glucose *in vitro* was studied In a number of diabetic patients the studies were carried out before treatment as well as at a time when satisfactory control from the clinical view point had been achieved by insulin therapy

Material and methods

All subjects were selected among diabetic and non-diabetic patients in hospital except four young healthy males who were studied ambulatorily All the patients were newly discovered cases and received no antidiabetic therapy before the investigation The standard diet of the hospital was given to almost all of them A few diabetics, one or two days before the study had received a diabetes diet of 1750 calories per day, containing 175 g of carbohydrates The non-diabetic subjects were so chosen that, with respect to age and weight to-height relationship they were comparable with the diabetic patients The selected material consisted of four groups with eight to ten subjects in each

Group 1 N Nine 'juvenile' non diabetic males whose ages ranged between 17 and 35 years, all lean or non obese One patient had lumbago, two had had myocarditis four to six months earlier and were hospitalized for circulatory studies The rest were in good health before and on the day of investigation

Group 2 D The juvenile diabetic subjects were nine males between 18 and 34 years of age all lean or non-obese On admission to hospital they were all in a mild or moderate ketotic state Four patients with acidosis were given 3 g of sodium bicarbonate orally per day before the investigation None of the patients had an acute infection They were all found to be insulin-dependent and insulin sensitive.

Group 3 N Eight 'adult' non-diabetic males whose body weight was above the mean 'ideal' (31) and whose ages ranged between 45 and 64 years Three patients were in hospital because of duodenal ulcer or irritable colon, one had mild hypertension, one had had a mild pneumonia three weeks earlier, one had aortic valvular stenosis without congestive failure, one had mild achalasia of the cardiae without weight reduction, and in one patient obesity was the only abnormality Two patients had in addition slightly abnormal electrocardiograms indicating coronary disease without subjective symptoms or evidence of recent myocardial infarction In none of the patients were signs of hepatic, renal, or endocrine disorder observed

Group 4 D Ten diabetic subjects of adult' type whose ages ranged between 38 and 75 years, all but one had a body weight above the mean ideal' (31) In three patients mild ketonuria was noted Treatment with sulphonylurea was indicated in all patients and for an observation period of up to fifteen months adequate diabetic control was attained by this therapy In three patients the electrocardiographic pattern indicated a coronary insufficiency without evidence of myocardial infarction

The diagnosis of diabetes mellitus was ascertained by 1 typical symptoms, 2 fasting glucose level in capillary blood exceeding 200 mg/100 ml, and 3 permanent glycosuria Moreover, Cushing's syndrome, phaeochromocytoma, acromegaly, and pancreatic insufficiency were excluded in all cases because the following laboratory tests were normal urinary 24-hour 17 ketosteroids 17 hydroxysteroids and catecholamines, field of vision lipid analysis of faeces, and roentgenograms of sella turcica and the pancreas. These studies were performed around one week after treatment was started

Acetone and acetoacetic ac d in urine were determined by the rout ne method of the hospital The procedures for the b opsy techn que incubat on method chem cal and radioact ve assays have been described n deta l elsewhere (45)

Calculat ons

Statistical calculat ons were made as recommended by Snedecor (35) The statist cal calculat ons of plasma triglycerides were performed after logarithm c transformat ons for reasons prev ously given (7) From the changes in the concentrat on of FFA and the amount of rad oactivity in med um during incubat on the method of Dole (12) was used to calculate the mean spec fic act ty of med um The FFA uptake into t ssue was calculated by d vid ng the rad o-act ty recovered n t ssue w th the mean spec fc act v ty of med um FFA The FFA ou put was calculated from the uptake and the net release of FFA s nce output equals net release plus uptake FFA esterif cat on index used as an est mate of the esterifica tion rate was calculated as the rat o between the rad oact vity recovered n the neutral t ssue l pid fract on and the total tissue rad oact ty In all these calculat ons the d fference between the initial activity in med um and the act v ty recovered within t ssue was used as a measure of the final med um act vity The var ation in metabolic activity of tissue samples incubated n dupl cate (45) was in this series of invest ga t ons for glucose uptake 20 1 % glycerol release 23 8 % FFA uptake 11 6 % FFA output 16 7 % FFA esterificat on index 5 1 % when the metabolic activity was calculated per unit of wet we ght Similar figures were obta ned when the metabolic act vitv was calculated per un t of neutral l p d weight of ad pose tissue The results here presented are based on the calculations where the metabol c parameters were ex pressed per un t of wet we ght The same differences between the clin cal groups studied were obta ned f om the calculations where the metabol c parameters were expressed per un t of neutral l p d we ght of adipose t ssue

Results

Arter al concentrat ons of plasma l p ds and plasma glycerol at t me of b opsy

Table II shows the mean arterial concentrat ons of FFA glycerol tri

ous ad pose ssue from d abe c and non-diabe c subjects n v tro Mean ± S E

FFA uptake (µEq g 2 hrs)		In al tissue FFA (µEq g)	Net changes of issue FFA (µEq g 2 hrs)	
KHA med um	EDTA plasma		KHA med um	EDTA plasma
1 43 ± 0 14	1 37 0 14	1 80 0 20	0 68 0 16 (<0 005)	0 59±0 14 <0 005)
0 49 0 10	0 83 0 07	2 31 0 44	0 83±0 51 >0 05	0 57±0 32 (>0 05
0 005	0 005	0 05	0 05	>0 05
0 40 0 15	0 51 0 08	1 97 0 43	0 0 ±0 7 (>0 05	0 66±0 44 (>0 05)
0 51 0 08	0 47±0 10	1 92 0 [illegible]5	0 11±0 22 (>0 05)	0 14±0 12 (>0 05
0 0	>0 05	>0 05	>0 05	>0 05

uptake
subjects.

TABLE II Arterial levels of plasma lipids and plasma glycerol in diabetic and non-diabetic subjects at removal of subcutaneous fat portions by surgical biopsy Mean ± S E.

Group	No of subjects	FFA (mEq/l)	Glycerol (mMol/l)	Triglycerides[1] (mMol/l)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)
J N	9	0 54±0 08	0 069±0 007	0 94 (0 67—1 31)	199 2± 6 0	194 8± 7 5
J D	9	0 99±0 14	0 115±0 016	2 79 (1 23—6 34)	325 0±69 7	295 4±41 8
p		<0 025	<0 05	<0 025	>0 05	>0 05
A N	8	0 74±0 04	0 108±0 008	1 40 (1 21—1 62)	249 5±22 7	243.3±13 8
A D	10	0 68±0 04	0 088±0 024	1 48 (0 99—2 20)	261 5±15 1	251 6±13 3
p		>0 05	>0 05	>0 05	>0 05	>0 05

[1] Mean and 95 % range calculated on logarithmic values, also used for the statistical calculations

Analytical methods

Glycerol (44), FFA (13, 40) glucose (30), and amount of radioactivity in the medium were determined as previously described (45) The content of FFA and the amount of radioactivity recovered as neutral lipids were determined as described in detail previously (45) In most experiments the neutral lipid content of the tissue samples was also determined gravimetrically Blood glucose, plasma FFA and plasma glycerol were analysed by the same methods as used for the incubation medium Cholesterol was determined by the Sperry and Webb technique (36), triglycerides by the method of Carlson and Wadstrom (9) as modified by Carlson (8), and phospholipids by estimation of inorganic phosphate (27)

TABLE III FFA output, FFA uptake, and net changes in tissue FFA during incubation of subcutane-

Group	No of subjects	FFA net release (μEq/g/2 hrs)		FFA output[1] (μEq g/2 hrs)	
		KHA medium	EDTA plasma	KHA medium	EDTA plasma
J N	9	1 76±0 27	1 45±0 25	3 19±0 39	2 82±0 34
J D	9	3 40±0 57	3 75±0 38	3 89±0 57	4 58±0 33
p		<0 05	<0 001	>0 05	<0 005
A N	8	2 51±0 62	2 21±0 71	2 91±0 61	2 72±0 73
A.D	10	3 84±0 37	3 31±0 51	4 35±0 38	3 78±0 50
p		>0 05	>0 05	>0 05	>0 05

[1] Calculated according to the formula devised by Dole (12) FFA net release – FFA output – FFA

[2] In parantheses are given the significance (p) of the difference between tissues within the group of

TABLE IV Incorporation of labelled palmitate into neutral lipids in per cent of total radioactivity recovered after incubation in subcutaneous fat portions of diabetic and non-diabetic subjects Mean ± S E

Group	No of subjects	Incorporation of labelled palmitate into neutral lipids (% of total tissue radioactivity) KHA medium	EDTA plasma
J N	9	93 9±1 3	92 3±1 3
J D	9	57 8±6 9	49 0±7 3
p		<0 001	<0 001
A N	8	75 1±3 5	70 3±4 0
A D	10	72 0±4 0	62 2±2 3
p		>0 05	>0 05

TABLE V Glycerol release from subcutaneous adipose tissue of diabetic and non diabetic subjects in vitro Mean ± S E

Group	No of subjects	Glycerol release (μMol g/2 hrs) KHA medium	EDTA plasma
J N	9	1 30±0 28	0 84±0 23
J D	9	2 34±0 55	2 09±0 45
p		0 05	<0 05
A N	8	1 25±0 37	1 09±0 21
A D	10	1 47±0 36	1 83±0 77
p		0 05	0 05

plasma $p < 0\,001$) (*table III* and fig 1) In the KHA medium this seems to be explained mainly by lower uptake of FFA ($p < 0\,001$) into tissue of the J D since no difference in the output of FFA from tissue was demonstrated between the groups However, in the EDTA plasma an increased output of FFA ($p<0\,005$) together with a lowering of the uptake of FFA ($p<0\,005$) would account for the greater net release of FFA in the J D On the assumption that after preincubation the concentration of FFA would be equal in all tissue samples of one subject, the net changes in tissue FFA were calculated as the difference in concentration of FFA between non incubated and incubated tissues The content of FFA within tissue of the J N decreased significantly by about one third in each type of medium ($p<0\,005$ both), while no consistent changes were observed in the J D

No significant differences were demonstrated between the A D and A N even if a tendency towards greater net release and output of FFA was noticed in the former group being most apparent during incubation in the KHA medium Comparison between the two non diabetic groups showed a significantly greater ($p < 0\,001$) uptake of FFA into the tissue of the J N

Incorporation of labelled FFA into neutral tissue lipids

Table IV shows the relative amount in per cent of total tissue radioactivity recovered as neutral lipids In tissue of the J N more than 90 per cent of total activity was recovered as neutral lipids, whereas in the J D only 50 to 60 per cent was found in this fraction (fig 1) the difference was highly significant in each medium ($p < 0\,001$) No significant differences were noticed between the A N and A D In each medium

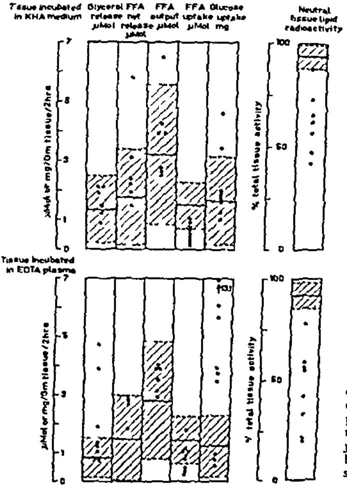


Fig. 1. Individual values of the metabolic parameters studied in adipose tissue samples of "juvenile" insulin-dependent, male diabetics during incubation in KHA medium and EDTA plasma. The mean values are given for tissue samples of non-diabetic male subjects (J. N.) and the hatched areas on both sides of the mean represent two standard deviations.

glycerides, cholesterol, and phospholipids in plasma of the "juvenile" group (J. D.), the "adult" diabetic group (A. D.) and their corresponding controls (J. N.) and (A. N.). Significantly higher plasma concentrations of FFA ($p < 0.025$), glycerol ($p < 0.05$), and triglycerides ($p < 0.025$) were observed in the J. D. compared with the J. N., while no differences were observed between the two old obese groups A. D. and A. N. The concentrations of FFA and glycerol were significantly higher ($p < 0.05$ both) in the old obese non-diabetic group than in the J. N. No difference in the concentrations of cholesterol and phospholipids were observed between the four groups. In two juvenile patients hypercholesterolemia (466 and 836 mg/100 ml), and hyperphospholipidaemia (554 and 435 mg/100 ml were noticed in addition to elevated triglycerides (36.2 and 4.34 mMole/l). Normalization of these lipid values was later attained during insulin therapy.

Changes in the concentration of FFA in medium and tissue during incubation

The net release of FFA from adipose tissue of the J. D. was significantly greater than from tissue of the J. N. during incubation in the KHA medium ($p < 0.05$) as well as in the EDTA

groups when taken together and compared with J N + J D. During incubation in the EDTA plasma the uptake of glucose appeared to be significantly greater in the J D than in J N ($p < 0.05$) (fig 1). It will be remembered that the content of glucose in plasma of the J D was about three fold that in plasma of the J N. A somewhat greater uptake of glucose from this type of medium was also observed in the A N in comparison with the A D, the difference was not s gn ficant

TABLE VII Glucose uptake into subcutaneous adipose t ssue of diabetic and non diabet c subjects in vitro Mean ± S E

Group	No of subjects	Glucose uptake in KHA medium (mg g/2 hrs)	Glucose uptake in EDTA plasma (mg g/2 hrs)
J N	9	1 64±0 25	1 24±0 18
J D	9	2 00±0 41	4 96±1 50
p		>0 05	<0 05
A N	8	1 04±0 35	1 31±0 75
A D	10	1 23±0 43	2 13±0 59
p		>0 05	>0 05

TABLE VIII Effect of increas ng glucose concentration in KHA med um on the net release of FFA from and the uptake of labelled palmitate by subcutaneous ad pose tissue of diabet c and non-diabet c subjects n v tro Mean given for dupl cate incubat ons in KHA med um

Case No	Classification	Medium glucose added (mg ml)	Glucose uptake (mg g 2 hrs)	FFA net release (µEq g 2 hrs)	FFA output (µEq g 2 hrs)	FFA uptake (µEq g 2 hrs)	Radioactivity incorporated (c p m./g) Neutral lipids	FFA	Neutral lipid radioactivity (% total tissue activity)	Specific activity of FFA (c p m µEq)
1	J N	1	1 05	2 82	4 46	1 64	194 900	13 500	93 5	3 940
		3	2 57	2 30	4 29	1 99	233 100	7 300	97 0	2 570
	A N	1	1 21	1 82	2 48	0 66	134 500	9 100	93 7	8 130
		3	2 11	1 37	2 61	1 24	241 400	23 300	91 2	2 330
3	A N	1	1 57	6 15	6 48	0 33	66 500	13 700	82 9	5 500
		3	3 72	4 10	4 58	0 48	75 500	15 000	83 4	6 690
4	J D	1	1 53	2 24	2 72	0 48	75 700	61 700	55 0	4 820
		3	3 72	1 9	2 25	0 39	61 00	41 900	40 9	6 090
	A D	1	1 27	5 76	6 17	0 41	29 500	10 200	74 3	2 240
		3	2 15	3 68	4 04	0 36	26 400	10 200	72 1	2 760
	A D	1		3 55	3 87	0 32	45 200	8 300	84 5	5 420
		3	3 43	3 72	4 02	0 30	43 900	10 700	86 4	7 590

Calculated by the formula devised by Dole (12)

TABLE VI. Comparison of fatty acid metabolism in human subcutaneous adipose tissue incubated in KHA medium and EDTA plasma, respectively. Mean ± S.E. between paired tissue sections incubated in KHA medium (I) and EDTA plasma (II), when calculated (I)−(II). In parentheses is given the significance (p) of the differences between tissues within the group of subjects when incubated in KHA medium and EDTA plasma

Group	No. of subjects	Glycerol release (μMol/g/2 hrs)	FFA output[1] (μEq/g/2 hrs)	FFA uptake[1] (μEq/g/2 hrs)	Neutral tissue lipid radioactivity (% total tissue activity)
J.N.	9	+0.45±0.13 (<0.01)	+0.37±0.16 (<0.05)	+0.06±0.14 (>0.05)	+1.6±1.1 (>0.05)
J.D.	9	+0.25±0.53 (>0.05)	−0.69±0.27 (<0.05)	−0.38±0.09 (<0.005)	+9.0±3.7 (<0.05)
p		>0.05	<0.005	<0.025	>0.05

[1] Calculated by the formula devised by Dole (12).

the incorporation of FFA into neutral lipids was significantly greater in the J.N. than in A.N. ($p < 0.001$).

Release of glycerol from adipose tissue into medium

From table V it appears that the mean release of glycerol in the KHA medium as in the EDTA plasma was greater from tissue of the J.D. than from tissue of the J.N. (fig. 1). The difference was significant, however, ($p < 0.05$) only in the EDTA plasma. A similar trend was observed in the A.D. compared with the A.N., though, in neither type of medium was the difference significant.

Comparison of the fatty acid metabolism in tissues incubated in KHA medium and EDTA plasma

Table VI presents the mean individual difference of glycerol release, FFA output and FFA uptake between tissues of the J.N. and J.D. incubated in the KHA medium and the EDTA plasma respectively. Glycerol release ($p < 0.01$) and FFA output ($p < 0.05$) from tissue of the J.N. were significantly greater when the fat pads were incubated in KHA medium whereas the FFA uptakes did not differ significantly between the media. On the other hand the uptake of FFA into tissue of the J.D. was significantly greater ($p < 0.005$) during incubation in the EDTA plasma whereas the FFA output was higher into this type of medium. The percentage of total tissue activity recovered as neutral lipids was similar in the media for the tissue of the J.N., whereas a higher percentage of neutral lipid activity was seen in tissue of the J.D. during incubation in KHA medium.

Uptake of glucose into adipose tissue

The uptake of glucose from the KHA medium, containing 1 mg/ml of glucose, was similar for all groups (table VII). A tendency towards lower uptake ($p < 0.1$) was observed in the two old obese

subjects before and during insulin treatment. Mean given for duplicate incubation in KHA medium

Glucose uptake (mg/g ? hrs)	Glycerol release (μMol/g/2hrs)	FFA net release (μEq/g/2 hrs)	FFA output (μEq/g/2 hrs)	FFA uptake (μEq/g/2hrs)	Neutral tissue lipid radioactivity (% total tissue activity)
0 67	1 89	2 24	2.39	0 15	15 2
0 44	1 76	2 27	1 85	0 42	50 2
3 48	0 63	1 49	2 42	0 93	83 4
2.24	0 41	1 69	2 55	0 86	78 4
1 69	6 30	3 62	3 91	0 29	21 8
1 96	1.26	1 98	2 49	0 51	47 1

required small doses of long acting insulin, as an unsatisfactory clinical effect had been obtained by sulphonylurea therapy alone

The metabolic activity of adipose tissue removed from each subject at different diabetic stages was studied by incubating duplicate fat portions in a KHA medium All tissue samples removed during a period of good clinical control showed a lower release of glycerol while the net release of FFA did not differ consistently from that before treatment A greater amount of labelled palmitate was taken up by tissues removed during insulin treatment in the two patients who had ketosis *initially* Simultaneously an increased incorporation of activity was observed in the neutral lipid fraction In the less severe case 2 slightly greater amount of labelled FFA was taken up during insulin treatment but in relative terms the incorporation of radio-palmitate into the neutral lipids was unchanged No consistent changes in the uptake of glucose were observed after the treatment

The individual values of the fatty acid metabolic parameters and the glucose uptake in the tissue samples of the J D are seen in fig 1 The corresponding mean values (± 2 S D) are given for tissues of the J N The most apparent changes in the tissues of the J D were in both types of media the lower percentage of total tissue activity recovered as neutral lipids Less marked alterations were the greater glycerol release and FFA output into EDTA plasma and the lower FFA uptake from KHA medium and EDTA plasma

Discussion

The present study shows that there is an increased net release of FFA *in vitro* from adipose tissue of human diabetics which is in conformity with previous observations (10) The increased net release of FFA seems to be due mainly to a reduced uptake of FFA and a concomitantly decreased incorporation of labelled FFA into the neutral tissue lipids These findings were the most apparent in tissue removed from insulin

TABLE IX. Comparison of FFA metabolism in vitro by subcutaneous adipose tissue of diabetic

Case No	Classification	Treatment	Arterial levels: Blood glucose (mg/100 ml)	Plasma glycerol (mMol/l)	Plasma FFA (mEq/l)	Ketonuria
1	AD—K	0	243	0.114	1.08	+++
		+	165	0.078	0.62	−
2	JD	0	425	0.085	0.75	−
		+	258	0.105	0.53	−
3	JD	0	388	0.169	2.34	+++
		+	130	0.092	0.47	−

Effect of increasing medium glucose concentration on net release and net uptake of FFA

In some experiments fat portions of one subject were incubated in duplicate in two KHA media containing different concentrations of glucose, 1.0 and 3.0 mg/ml medium, respectively. Five of the subjects (table VIII) had been included in the previous studies. Case 3, a 64-year-old non obese man, was excluded from these, since he had a K value of 1.07 per cent per minute in the glucose tolerance test, a history of chronic alcoholism, and low concentrations of plasma cholesterol (197 mg/100 ml) and plasma phospholipids (179 mg/100 ml). In the medium with higher glucose concentration a decrease in the net release of FFA was observed in almost all the cases. A greater amount of radio-palmitate was taken up by the tissue of the non diabetic subjects, while the uptake by tissue of diabetic patients was similar at both concentrations of glucose. The ratio between neutral lipid activity and total tissue activity was unaltered in all the subjects. No consistent change in the specific activity of tissue FFA was observed in the diabetics, whereas a decrease was obvious in tissue of definitely non diabetic individuals.

Effects of insulin treatment on FFA metabolism of adipose tissue

Three diabetic patients were studied on two occasions, namely before treatment was started and after one to three months' treatment with insulin had led to satisfactory clinical control of the diabetic state. At the first study two patients had moderate ketosis (table IX), and in all of them the concentrations of plasma FFA and blood glucose were higher than at the second investigation performed more than 24 hours after the last injection of intermediate acting type. Case 1 was not included in the previous studies, since he was an old, obese man who initially had moderate ketosis necessitating insulin treatment but was later maintained on sulphonylurea and finally on diet alone. Case 2 had a diabetes which

of FFA for the triglyceride synthesis Moreover, an augmented breakdown of *de novo* synthetized, and hence labelled, triglycerides might lead to an increased amount of activity in the FFA pool In the present study, however, it seems rather unlikely that, judged from the glycerol release, the moderate increase in the triglyceride breakdown would provoke any great accumulation of unlabelled and labelled FFA in the precursor pool of FFA Moreover the incorporation of labelled palmitate into neutral tissue lipids of diabetics was only slightly lower during incubation in EDTA plasma, when an increased tri glyceride breakdown was observed Under the conditions used, it seems reasonable to assume that the ratio between radioactivity recovered as neutral lipids and total tissue activity gives a certain measure of the esterifica tion of labelled FFA into neutral tissue lipids i e tri and diglycerides The decreased incorporation of labelled FFA into neutral tissue lipids of human dia betics might then indicate a decrease in the esterification of FFA

Glucose uptake

The present investigation shows that glucose uptake from the KHA medium was similar into tissues of non diabetic and diabetic subjects Thus the results are in accordance with previous studies on human subcutaneous adipose tissue (10 15) They also correspond with experiments on epididymal fat pad of pancreatectomized rats showing an uptake of glucose similar to that of normal rat tissue (14) At variance with the results it has been preliminary reported that slices of omental tissue removed from diabetic patients take up glucose less rapidly than omental tissue of normal subjects (22) Of special interest in this connection are the recently reported experiments on glucose uptake by adipose tissue from rats treated with anti insulin serum (38) They demonstrate a diminished glucose uptake into tissues removed 30 minutes after the injection of anti insulin serum, but a normal glucose uptake into tissue removed one and three hours after the administra tion These results indicate that uptake of glucose into rat adipose tissue is significantly impaired only when the insulin-deficiency is almost total

During incubation in the EDTA plasma there was a *significantly greater* uptake of glucose into tissue of diabetic patients than into tissue of non-diabetic subjects This might be due to the higher concentration of glucose in the plasma media of the diabetics since the uptake of glucose by human sub cutaneous adipose tissue is related to the medium concentration of glucose (45) whereas its response to insulin added *in vitro* is small (15, 32) The present study shows that the uptake of glucose into tissue of normals was similar from EDTA plasma known to contain a low insulin like activity in fasting state and from KHA medium

Relationship between glucose uptake and fatty acid metabolism in adipose tissue

The present investigations have shown that an increased release of FFA and glycerol as well as an impaired esterifica tion of FFA exist in diabetes under conditions when the uptake of glucose

dependent diabetics In this type of diabetes an increase in the release of glycerol and output of FFA was also demonstrated, the latter consequently contributing to the increased net release of FFA In tissue of "adult" obese diabetics a tendency towards similar disturbances was seen There was no significant difference, however, in any of the parameters studied between tissues of "adult" obese diabetic and "adult" obese non-diabetic subjects Therefore the subsequent discussion will be restricted mainly to the observations made in tissue of insulin-dependent diabetics, divided under three headings 1 fatty acid metabolism, 2 glucose uptake and 3 relationship between glucose and fatty acid metabolism

Fatty acid metabolism

In these experiments the release of glycerol from tissue into medium has been assumed to reflect the complete breakdown of the tissue triglycerides This seems reasonable since firstly, the utilization of glycerol by adipose tissue *in vitro* is poor (6, 28, 34) except under special conditions (4) Secondly, it has been shown recently that the glycerol level within tissue does not change appreciably under the conditions used (45) Moreover, in accordance with recent observations (46), the present data indicate that the changes in release of glycerol and output of FFA were related to each other Thus the output of FFA and the release of glycerol were similar from tissue of non diabetic and diabetic subjects during incubation in KHA medium, whereas the output of FFA as well as release of glycerol were greater from tissue of diabetic subjects during incubation in EDTA plasma These findings might indicate that in plasma of diabetic subjects there was a shift towards increased lipolytic or decreased antilipolytic activity Support for this concept is the observation that, when incubated with adipose tissue from fed rats, serum of untreated diabetic subjects contains a higher FFA releasing activity than serum of normal subjects (33) Furthermore the present observations seem to correspond well with the findings of increased concentrations of FFA as well as glycerol in plasma of insulin-dependent diabetics From several reports reviewed elsewhere (11, 19) there is evidence that various hormones, i e growth hormone and glucocorticoids, are responsible, at least partly, for the increased mobilization of FFA from the fat depots in diabetic state On the other hand it was recently demonstrated that insulin acts as an antilipolytic in the absence of glucose (21, 29), for which reason an insulin deficiency might lead to an enhanced lipolytic state in adipose tissue

The most apparent changes in tissue of the diabetic subjects were a decrease in the uptake of FFA and, in absolute as well as in relative terms, a decrease in the incorporation of labelled palmitate into the neutral tissue lipids It may be pointed out that a decreased incorporation of labelled palmitate into the neutral lipids does not simply indicate a decrease in the synthesis of the triglycerides (37, 41) Similar results may also be obtained in lipolytic states, when unlabelled FFA formed by the lipolysis dilute the activity in the precursor pool

Of course it cannot be decided whether these differences are related to a disparity in age of the groups or to a difference in body fat

Summary

The fatty acid metabolism was studied *in vitro* on subcutaneous adipose tissue obtained by surgical biopsy from subjects with diabetes mellitus of juvenile and adult obese type The juvenile type diabetics had, in comparison with the control subjects, an increased release of FFA and glycerol from adipose tissue They had also significantly elevated concentrations of FFA, glycerol and triglycerides in plasma The fatty acid metabolic defects behind the net release of FFA from adipose tissue in juvenile type diabetes consisted of two different defects 1 decreased assimilation and esterification of FFA, and 2 increased breakdown of the tissue triglycerides There was no evidence for an impaired uptake of glucose into tissue of juvenile type diabetics incubated in albumin medium The fatty acid metabolic defects were observed also when adipose tissue of these patients were incubated in plasma obtained at the biopsy and when the uptake of glucose was higher than into adipose tissue of the control subjects

No apparent fatty acid metabolic changes were observed in adult-obese type diabetics in comparison with the old obese control subjects neither in the metabolism of adipose tissue *in vitro* nor with respect to the concentrations of FFA and glycerol in plasma The rate of glucose uptake into adipose tissue *in vitro* was unimpaired also in this type of diabetics

The deteriorated uptake and esterification of FFA in adipose tissue of juvenile type diabetics were in part restored during insulin treatment

The mechanisms for the impairment of the synthesis and the breakdown of triglycerides in adipose tissue of juvenile-type diabetics were discussed in relation to the results

Acknowledgements

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is equal to or higher than in tissue of non-diabetic subjects Thus the results agree with studies of adipose tissue of alloxan-diabetic rats, where an increased uptake of glucose was obtained by addition of insulin *in vitro* while the net release of FFA was unaffected (3) In addition it has been shown in adipose tissue of anti insulin serum treated rats that the net release of FFA was increased even when the uptake of glucose was normal (38) Since α-glycerophosphate, the obligate precursor of glyceride-glycerol, is assumed to emanate mainly from the glucose metabolism (6, 28), the following alternatives may be considered in explaining why an elevated release of FFA and a decreased uptake of FFA occur simultaneously with an increased uptake of glucose Firstly, it is conceivable that the production of α-glycerophosphate is reduced in diabetic tissue in spite of an unimpaired uptake of glucose No data have hitherto been reported on the glyceride-glycerol formation from glucose by adipose tissue of human diabetics However, in adipose tissue of rats made diabetic by alloxan or anti-insulin serum, it has been shown that the glyceride-glycerol production was fairly well preserved in comparison with the fatty acid synthesis (5, 6, 38) Secondly it is suggestible that the reduced esterification is not, or only in part caused by an insufficient supply of α glycerophosphate An impaired esterification might just as well be due to a decreased activation of FFA on account of the lack of ATP in the responsible thiokinase system Of special interest in this connection are the experiments showing that the production of ADP and ATP by heart homogenate of alloxan-diabetic rats is decreased even if the utilization of glucose is normal (17)

By addition of an increasing concentration of glucose to the medium an increased uptake of radio-palmitate into tissue of non diabetic subjects was obtained, whereas the percentage of total tissue activity recovered as neutral lipids remained unaltered This finding is in accordance with similar studies of isolated adipose tissue of normal rats (1) and might indicate that the fractional esterification had reached and remained at a maximal level In tissue of diabetic subjects neither the net uptake of FFA nor the relative amount of activity in the neutral lipid fraction altered at increased glucose concentration and hence increased uptake of glucose By correction of the diabetic state with insulin therapy an increased net uptake of FFA and greater incorporation of activity as neutral lipids were demonstrated, whereas the uptake of glucose *in vitro* was unaltered The most plausible explanation of this effect might be an insulin dependent stimulation of the production of ATP and/or α glycerophosphate *in vivo*, although other mechanisms are, of course, possible

Differences in the fatty acid metabolism of adipose tissue were also seen between the two non diabetic groups of subjects Thus the uptake of FFA and the esterification of FFA were lower in adipose tissue of the old obese individuals During the incubation the concentration of FFA decreased significantly in tissue of young non diabetics, but remained unaltered in tissue of old obese subjects

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The Effect of Varying Pacemaker Rate on Physical Work Capacity in Patients with Complete Heart Block

By

IRMA ÅSTRAND and JOHAN LANDEGREN

During the last few years the techniques for long term artificial cardiac pacemaking have been continuously improved. Apparently the primary goal was the prevention of Adams Stokes attacks due to asystole although it was early found (14) that prophylaxis of attacks due to paroxysmal ventricular fibrillation in complete heart block could also be successfully achieved by augmentation of the basic rhythm.

Along with the improvements in techniques the indications have then been extended to other chief manifestations of complete heart block, i.e. cardiac insufficiency resulting from the slow ventricular rate and probably more important the subnormal acceleration of heart rate on exertion.

Most workers use pacemakers with a constant stimulation frequency in the order of 60—75—90 imp/min. A constant rate however affords only a partial correction of the functional disturbance resulting from complete heart block. Several ways for achieving variability of the pacemaker rate are at hand. For reviews of the various principles employed, the reader is referred to Nathan et al. (10).

Apparently such devices being available, the problem is whether the advantages from rate variability are large enough to motivate the adoption of such more complicated methods.

Several workers have paid attention to these problems but the opinions and conclusions differ widely and no definite answers have been given (4, 7, 12, 15). One reason for this is certainly that most investigations have been restricted to rest or light work.

In order to obtain a better understanding of the problems mentioned, 7 patients with artificial pacemakers were studied at 3 different stimulation rates during several work loads on a bicycle ergometer.

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9th International Congress of Internal Medicine, 7th—10th September 1966, Amsterdam (Holland)

Chairman of the Congress Professor J Mulder, M D

Principal subjects of the Congress

1 Integration in internal medicine

2 Auto immune diseases and transplantation

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4 *Radio-isotopes* and radiodiagnostics

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Secretariat 9th International Congress of Internal Medicine, c/o Holland Organizing Centre, 16, Lange Voorhout, The Hague, Holland

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The 6th European Congress of Allergology will be held in Stockholm September 5th—9th, 1965

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Principal subjects of the Congress

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2 The importance of inhalation tests in the etiological diagnosis of allergic diseases of the bronchi and in evaluation of the effects of specific hyposensitization

3 Contact eczema in theory and in practice

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The 3rd International Congress of Nephrology will be held September 25—30, 1966, Washington, D C, U S A

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Secretariat Third International Congress of Nephrology, 9650 Wisconsin Avenue, Washington, D C 20014 U S A

done approximately 15 min before the start of the experiment, and between morning and afternoon examinations it was readjusted to its usual medium value

In each separate work procedure in the morning and in the afternoon the patient first cycled for 6 min at a low work load 200 or 300 kpm/min The load was then increased to 400—600 kpm/min Should this also be tolerated for 6 min the load was increased once more The test was interrupted either when subjective troubles became intense enough to prevent the patient from continuing cycling or if multiple ventricular ectopic beats appeared

During the last 2 min of each work period of 6 min expired air was collected in Douglas bags for measurement of pulmonary ventilation and for calculation of oxygen uptake (oxygen content was determined according to the Haldane technique) Should the patient not be able to accomplish work for 6 min the air collection was made earlier and for a shorter period of time

Lactic acid estimations were performed on samples of capillary blood collected from the finger tip just prior to the termination of each work load and besides this approximately 2 and 4 min after the last load The analysis was performed according to a modification (13) of the Parker and Summerson method (3) The peak value for the last load was used irrespective of whether the sample was taken during or after work

The electrocardiogram was recorded at rest and *continuously* during work by a 4-channelled direct ink writing electrocardiograph (Elema with four chest leads the reference electrode being placed on the forehead (CH leads) In order to make the electric stimuli from the pacemaker identifiable the muscle tremor suppression filter of the electrocardiograph was connected (reducing the upper cut-off frequency to about 40 c s)

After each test the patient was asked to describe the subjective experiences during the work and particularly those troubles that had been the immediate cause of interruption of the cycling

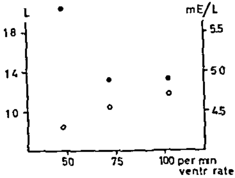


Fig 1 Maximal oxygen intake ($\dot{V}O_2$ l ○) and blood lactate concentration (●) at three different pacemaker frequencies in case 1

At the morning tests oxygen uptake was not determined but the patient breathed through the valve in order to get used to the procedure Otherwise the procedures at the morning tests corresponded with the afternoon tests i e with regard to work loads, work time ECG recording and scheme for collection of blood samples

Results

Table II summarizes the observations during work at each of the three pacemaker frequencies Blood lactate concentration atrial rate and work time are given as mean values of the morning and afternoon estimations these did not differ significantly

With the high pacemaker rate and on 2nd load all patients were able to proceed work for a longer period of time than with the low pacemaker rate Actually 3 of them could even work at a higher load The improvement of work capacity was mirrorred by a rise in oxygen uptake towards a more adequate oxygen supply in 5 cases while in the remaining 2 it was only slightly increased or

TABLE I Pertinent data concerning the seven cases (with acquired complete heart block)

Case	Sex	Age at invest (yrs)	Main indication(s) for pacemaker treatment[1]	Months after pacemaker application	Functional classification (N Y H A)[2] before pacemaker — in compl block	Profession/occupation: Before AV block	Profession/occupation: At time of invest
1	♂	14	AV III with very slow rate and numerous AS	10	IV	Schoolboy	Studies at home
2	♂	48	Changing AV block with AS moderately severe mitral stenosis	22	II	Furniture polisher	Light handicraft work
3	♂	58	AS of varying frequency, permanent AV III	4	II	Bookbinder	Bookbinder
4	♂	59	AV III, acute deterioration with numerous AS	8	III	Truck driver	Porter (school attendant)
5	♂	62	AV III with markedly reduced exercise tolerance	8	II	Farmer	Light activities in farm work
6	♀	63	Permanent AV III, sporadic AS, but of severe character	[illegible]	II—III	Housewife	Light activities in household
7	♂	72	Changing AV block with AS	36	II—IV	Tailor	Tailor

[1] AV III Complete atrioventricular block AS Adams-Stokes attacks
[2] N Y H A New York Heart Association

Material and methods

For the investigation 7 patients were selected, who had been supplied with external pacemakers (EM 138, manufactured by the Elema Schönander Co, Stockholm Sweden) for long term treatment of Adams Stokes attacks and/or cardiac insufficiency due to complete heart block One patient was treated with digitalis but no one with isoproterenol or other drugs that could influence the atrial rate response Some data on the patients are presented in table I

With the pacemaker set at three different frequencies, the patients worked on a bicycle ergometer The frequencies, which will be referred to below as "low", "medium", and "high", were 27—48 imp/min, 67—72 imp/min and 92—105 imp/min respectively The medium frequency corresponded to that ordinarily employed in the patient At each stimulation rate two exercise procedures were performed, one in the morning and one in the afternoon of the same day, thus altogether six procedures were performed by each subject *On the first* day the patient worked with the pacemaker set at the medium rate, on the 2nd at the high and on the 3rd at the low rate *Adjust*ments to the high and low frequencies were

done approximately 15 min before the start of the experiment and between morning and afternoon examinations it was readjusted to its usual medium value

In each separate work procedure in the morning and in the afternoon the patient first cycled for 6 min at a low work load, 200 or 300 kpm/min The load was then increased to 400—600 kpm/min Should this also be tolerated for 6 min the load was increased once more The test was interrupted either when subjective troubles became intense enough to prevent the patient from continuing cycling or if multiple ventricular ectopic beats appeared

During the last 2 min of each work period of 6 min expired air was collected in Douglas bags for measurement of pulmonary ventilation and for calculation of oxygen uptake (oxygen content was determined according to the Haldane technique) Should the patient not be able to accomplish work for 6 min the air collection was made earlier and for a shorter period of time

Lactic acid estimations were performed on samples of capillary blood collected from the finger tip just prior to the termination of each work load and besides this approximately 2 and 4 min after the last load The analysis was performed according to a modification (13) of the Parker and Summerson method (3) The peak value for the last load was used irrespective of whether the sample was taken during or after work

The electrocardiogram was recorded at rest and *continuously* during work by a 4-channelled direct ink writing electrocardiograph (Elema) with four chest leads the reference electrode being placed on the forehead (CH leads) In order to make the electric stimuli from the pacemaker identifiable the muscle tremor suppression filter of the electrocardiograph was connected (reducing the upper cut-off frequency to about 40 c/s)

After each test the patient was asked to describe the subjective experiences during the work and particularly those troubles that had been the immediate cause of interruption of the cycling

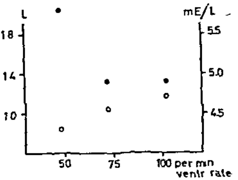


Fig 1 Maximal oxygen intake ($\dot{V}_{O_2}$ l ○) and blood lactate concentration (●) at three different pacemaker frequencies in case 1

At the morning tests oxygen uptake was not determined but the patient breathed through the valve in order to get used to the procedure Otherwise the procedures at the morning tests corresponded with the afternoon tests i e with regard to work loads, work time ECG recording and scheme for collection of blood samples

Results

Table II summarizes the observations during work at each of the three pacemaker frequencies Blood lactate concentration atrial rate and work time are given as mean values of the morning and afternoon estimations, these did not differ significantly

With the high pacemaker rate and on 2nd load all patients were able to proceed work for a longer period of time than with the low pacemaker rate Actually 3 of them could even work at a higher load The improvement of work capacity was mirrorred by a rise in oxygen uptake towards a more adequate oxygen supply in 5 cases while in the remaining 2 it was only slightly increased or

TABLE I. Pertinent data concerning the seven cases (with acquired complete heart block)

Case	Sex	Age at invest. (yrs)	Main indication(s) for pacemaker treatment[1]	Months after pacemaker application	Functional classification (N.Y.H.A.)[2] before pacemaker — in compl. block	Profession/occupation: Before AV block	Profession/occupation: At time of invest.
1	♂	14	AV III with very slow rate and numerous AS	10	IV	Schoolboy	Studies at home
2	♂	48	Changing AV block with AS, moderately severe mitral stenosis	22	II	Furniture polisher	Light handicraft work
3	♂	58	AS of varying frequency, permanent AV III	4	II	Bookbinder	Bookbinder
4	♂	59	AV III, acute deterioration with numerous AS	8	III	Truck driver	Porter (school attendant)
5	♂	62	AV III with markedly reduced exercise tolerance	8	II	Farmer	Light activities in farm work
6	♀	63	Permanent AV III, sporadic AS, but of severe character	5	II—III	Housewife	Light activities in household
7	♂	72	Changing AV block with AS	36	II—IV	Tailor	Tailor

[1] AV III = Complete atrioventricular block. AS = Adams-Stokes attacks.
[2] N.Y.H.A. = New York Heart Association.

Material and methods

For the investigation 7 patients were selected, who had been supplied with external pacemakers (LM 138, manufactured by the Elema Schönander Co., Stockholm, Sweden) for long term treatment of Adams-Stokes attacks and/or cardiac insufficiency due to complete heart block. One patient was treated with digitalis but no one with isoproterenol or other drugs that could influence the atrial rate response. Some data on the patients are presented in table I.

With the pacemaker set at three different frequencies, the patients worked on a bicycle ergometer. The frequencies, which will be referred to below as 'low', 'medium', and 'high', were 27—48 imp./min., 67—72 imp./min. and 92—105 imp./min. respectively. The medium frequency corresponded to that ordinarily employed in the patient. At each stimulation rate two exercise procedures were performed, one in the morning and one in the afternoon of the same day, thus altogether six procedures were performed by each subject. On the first day the patient worked with the pacemaker set at the medium rate, on the 2nd at the high and on the 3rd at the low rate. Adjustments to the high and low frequencies were

oxygen uptake l/min)

2nd load					3rd load							
Work load (kpm/min)	VO_2 (l)	Blood lact conc (mEq/l)	Work time (min)	Subj troubles*	Beats per min Ventr	Auric	Work load (kpm/min)	VO_2 (l)	Blood lact conc (mEq/l)	Work time (min)	Subj troubles*
400	0 85	5 8	1 8	2+4							
	1 04	4 9	4 0	2							
	1 05	4 3	6 0	4	102	187	600	1 17	4 9	1 3	4
450	0 98	4 1	3 5	1+2							
	1 07	4 3	6 0	2+4							
	1 11	3 3	6 0	4	100	162	600	1 52	4 7	2.2	4
600	1 24	3 1	3 0	2+4							
	1 35	4 2	4 0	2+4							
	1 43	3 5	4 0	4	100	144	750	1 65	4 0	1 7	2
600	0 98	4 7	1 5	1+3							
	1 34	3 2	3 0	2 [illegible]							
	1 54	3 0	6 0	2							
—		—									
600	1 00	4 2	1 5	2							
	1 28	3 8	2 5	2							
—											
400	0 85	7 7	6 0	2+4							
	0 93	6 4	6 0	2+4							
600		5 8	0 8	2							
	1 26	5 3	2 0	2 4							
	1 29	3 7	2 0	[illegible]							

creased on transition from rest to work and as the work loads were progressively increased The progressive augmentation of atrial rate occurred in a similar fashion as observed in normals i e it could be observed already during the first seconds of exercise The atrial rate at various loads corresponds to the heart frequencies that might be expected in untrained but healthy individuals 1

During work at the first load the atrial rate was lower in 4 of the subjects when the pacemaker frequency was elevated from low to medium and from medium to high In 2 other cases the same thing was observed at transition from low to medium in one case the atrial rate remained essentially unchanged The corresponding differences were much smaller on the 2nd load about 10 beats/

TABLE II Data obtained at rest and during work with three different pacemaker rates (VO_2(l) =

Case	Rest		1 st load							2 nd load	
	Beats per min		Beats per min		Work load (kpm/min)	VO_2 (l)	Blood lact conc (mEq/l)	Work time (min)	Subj troubles[3]	Beats per min	
	Ventr	Auric	Ventr	Auric						Ventr	Auric
1	48	112	48	163	200	0.65	4.1	6.0	0	48	180
	72	104	[1]72	144		0.63	3.9	6.0	0	[1]72	179
	102	110	102	140		0.71	2.1	6.0	0	102	172
2	43	103	[2]43	150	300	0.72	2.6	6.0	1+2	[2]43	159
	67	89	67	142		0.90	2.7	6.0	0	67	159
	100	91	100	122		0.90	2.0	6.0	0	100	141
3	[2]54	83	[2]54	111	300	0.92	1.6	6.0	0	[2]54	136
	70	84	70	113		1.09	2.2	6.0	0	70	137
	100	81	100	105		1.05	1.7	6.0	0	100	131
4	27	102	[1]27	154	300	0.90	2.8	6.0	1+3	[1]27	163
	72	88	72	110		0.90	1.8	6.0	0	72	139
	105	90	105	107		0.91	1.6	6.0	0	105	141
5	41	81	41	138	300	–	2.5	1.8	1+2	—	—
	68	83	68	119		0.73	2.8	6.0	0	68	137
	92	80	92	108		0.93	2.2	6.0	0	92	136
6	45	80	[2]45	124	200	--	3.6	6.0	5	—	—
	68	82	[1]68	110		0.65	2.3	6.0	0	[1]68	151
	97	80	97	111		0.69	2.7	6.0	0	97	154
7	42	88	[1]42	152	300	0.85	2.7	6.0	0	[1]42	163
	67	84	[1]67	136		0.87	2.8	6.0	0	[1]67	163
	105	81	[1]105	124		1.02	2.0	6.0	0	[1]105	153

[1] Occasional ventricular ectopic beats
[2] Frequent ventricular ectopic beats
[3] 1 dizziness 2 dyspnoea 3 nausea 4 stiffness in the legs 5 pain in the heart

unchanged The altered oxygen uptake was accompanied by a decrease in the blood lactate concentration (fig 1) The blood lactate concentration was significantly lower $(0.05 > P > 0.01)$ with the high stimulation rate compared to the medium at the second work load although the work time on several occasions was longer at the high rate

In 2 cases the atrial rate at rest was approximately 10 beats/min higher at the low pacemaker frequency than at the medium and high while it did not change significantly in the remaining 5 cases when the pacemaker rate was varied (table II)

In all cases — and each level of stimulation rate—the atrial rate was in

order was considered as a contraindication to a second test at the low stimulation frequency (15)

The main subjective trouble was the same at the morning and afternoon examination At the low pacemaker rate three patients experienced dizziness during work, one anginal pains, which occurred together with multiple ventricular ectopic beats At the other pacemaker rates the pervading symptom was dyspnoea (table II) In cases nos 1 and 6 examination at a lower pacemaker frequency than that reported in table II was originally intended Already in the sitting position they experienced such intense symptoms in the form of dizziness however, that exercise could not be performed

Discussion

The data obtained at the exercise tests, i e highest work load tolerated, time of work at that load, oxygen uptake, blood lactate concentration, subjective troubles and atrial rate uniformly indicate a better capacity for physical work with the high pacemaker frequency than with a low one This becomes apparent already at a low intensity of work, corresponding to 0 7—0 9 l O_2/min and it is still more marked at higher intensities The more adequate oxygen uptake and the smaller rise in blood lactate concentrations at similar work loads indicate a more adequate rise in cardiac output at higher ventricular rates (6)

These findings evidently are of great importance for the patients with regard to their professional work A constant pacemaker rate of, say, 75 imp/min certainly does not allow longer periods of time for such activities as require more than approximately 1 1 O_2/min This oxygen uptake is actually not high, it corresponds to the oxygen uptake during a walk at 5 km/h (3 1 mph)

One alternative would be setting the pacemaker at a higher but still constant, frequency, e g 100 imp/min Many patients, however, will experience palpitations and other symptoms of a more diffuse character at a high rate at rest Furthermore, the oxygen consumption of the heart is higher at higher rates at a constant cardiac output (5) which especially in these cases must be considered as an adverse effect The only advantage of a high stimulation rate at rest is probably its efficiency in prevention of ventricular fibrillation and other ventricular arrhythmias (9 15)

Therefore, the best alternative must be pacemakers which allow frequency regulation according to the varying physiological needs As atrial rate increases in these patients as the demands for oxygen in actively working muscles rise the ideal way of treatment should be the use of pacemakers in which the frequency of stimulus is dependent upon atrial activity P wave stimulators (10)

Even when this form of pacemaker treatment has reached a degree of security and dependability high enough to motivate its more general adoption determination of physical work capacity will be highly valuable in patients with atrio ventricular block This statement applies to all persons with a functional disability since it is desirable that the

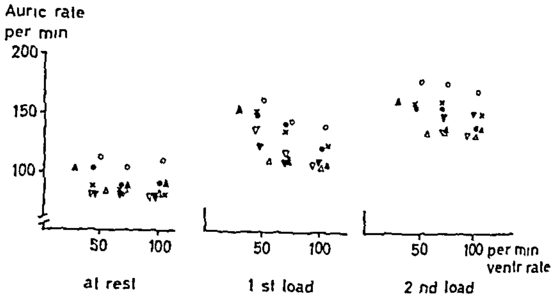


Fig 2 Auricular rates at three different ventricular rates at rest and during the 1st and 2nd work load The different symbols correspond to the 7 cases

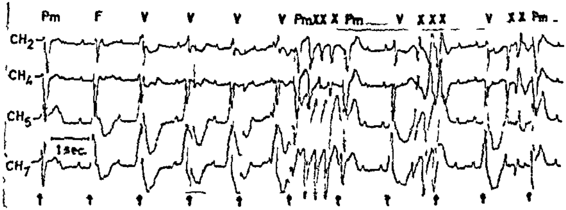


Fig 3 Multiple ventricular ectopic beats during work at a low pacemaker frequency (case No 6) The pacemaker (Pm) induces a basic rhythm (45 imp /min) but idioventricular beats (V) spontaneously occur regularly with a somewhat higher rate (about 50 beats min) Fusion beats (F) are also seen Additionally ventricular beats of varying configuration (X) can be observed occurring either in pairs or in series causing short runs of ventricular tachycardia It should be noticed that sometimes the multiple ventricular beats are initiated by the pacemaker but sometimes they occur without relation to the electric stimuli

min , in three of the patients when high and medium frequencies are compared (fig 2)

In all but one subject ventricular ectopic beats appeared mainly at the low pacemaker rate In case 3 they occurred regularly between each 2 paced beats For this reason the rate is given as twice the actual pacemaker rate in table II Numerous spontaneous (or ectopic) idioventricular beats were observed also in cases 2 and 6 The latter patient showed multiple ectopic beats (or short bursts of ventricular tachy cardia) (fig 3), and the grave prognostic significance of this kind of rhythm dis

4 BELLET S Clinical disorders of the heart beat Lea and Febiger Philadelphia 1963 p 1105
5 BERGLUND F BORST H G DUFF F & SCHREINER G L Effect of heart rate on cardiac work myocardial oxygen consumption and coronary blood flow in the dog Acta physiol scand *42* 185 1958
6 BEVEGÅRD S Observations on the effect of varying ventricular rate on the circulation at rest and during exercise in two patients with an artificial pacemaker Acta med scand *172* 615 1962
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11 PASSMORE R & DURNIN J V G A Human energy expenditure Physiol Rev *35* 801 1955
12 SOWTON G E Artificial cardiac pace making with particular reference to cardiac physiology Thesis University of Cambridge 1963 p 261
13 STROM G The influence of anoxia on lactate utilization in man after prolonged muscular work Acta physiol scand *17* 440 1949
14 ZOLL P M Resuscitation of the heart in ventricular standstill by external electric stimulation New Engl J Med *247* 768 1952
15 ZOLL P M & LINENTHAL A J External and internal pacemakers Circulation *28* 455 1963

demands during professional work should be lower than 50 per cent of the physical work capacity (1, 2, 8). This is illustrated by patients, reported on in this paper.

A. Case No. 5. Before developing a permanent complete heart block, this man actively worked as a farmer. Farm work often includes handling of heavy objects, and certain activities require an oxygen consumption of 1.5 l/min or more at ordinary speed of work (11). This patient, having a capacity for oxygen consumption of about 1.3 l/min (or somewhat more at the highest stimulation rate tested), cannot be expected to manage such activities. He may tolerate daily occupations, which require about 0.7 l O_2/min.

B. Case No. 3. The reverse situation is exemplified by this case. He has been able to resume his former professional work without limitations, while in complete heart block before pacemaker treatment, he had to leave certain jobs to his colleagues. He is a book binder, and the heavier moments in this occupation require about 0.5 l O₂/min. A paced heart rate of 70 beats/min (table II) makes possible an oxygen uptake of more than 1 l/min without undue accumulation of lactate, thus leaving a fairly good margin for his professional work performance.

Summary

Seven patients with artificial pacemakers were examined during work on a bicycle ergometer with the pacemakers set at three different frequencies, 27—48 imp./min., 67—72 and 92—105 imp./min.

Uniform results concerning maximum work intensity, work time, oxygen uptake, and blood lactate concentration were obtained, indicating an increased physical work capacity at higher stimulation rates.

The atrial rate increased on transition from rest to work and at successively rising work loads, indicating that the best alternative for these patients must be pacemakers, the frequencies of which are dependent upon the atrial activity.

These results are discussed with particular reference to the patients' professional occupations.

Acknowledgement

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Addendum

Since the manuscript was prepared, Segel N., Hudson W. A., Harris P. and Bishop J. M. published a paper with the title "The circulatory effects of electrically induced changes in ventricular rate at rest and during exercise in complete heart block". J. clin. Invest. *43*: 1541, 1964. Results of the present study are in applicable parts in agreement with their observations.

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Cushing's Syndrome Associated with Chromophobe Adenoma of the Hypophysis

By

JÁNOS P RADÓ, JÓZSEF TAKÓ and GYORGY MIKLÓS

Earlier, the chromophobe adenomas of the hypophysis had been believed to be inactive endocrinologically but even in the original description of Cushing's syndrome there were three cases in which the adenoma of the pituitary was apparently chromophobe (2) Though 30 years have elapsed since then and innumerable reports have been published on Cushing's syndrome only a few new cases of chromophobe adenoma of the pituitary associated with Cushing's syndrome have been described (1—4 6—8 10 11)

It has therefore been suggested that also the chromophobe adenoma may be capable of secreting ACTH and thus may be active endocrinologically

The problem has gained new significance since serious intracranial complications have been observed in patients with Cushing's syndrome treated by adrenalectomy (9 12) The elevation of the blood level of ACTH following adrenalectomy has made it likely that an absence of cortical hormones (a so-called negative feedback mechanism) was responsible for the unrestricted growth of the hypophysial tumour (4)

In this paper we shall describe a case of Cushing's syndrome with a chromophobe adenoma demonstrated in the hypophysis As far as we know it the case is unique in that there was also an adrenal cortical adenoma besides the Cushing's syndrome and chromophobe adenoma

Case report

A female aged 56 years was admitted on January 21 1961 The childhood and familial histories were non-contributory First menstruation at the age of 15 years the last at the age of 44 She had been suffering from diabetes for 12 years and had been treated with insulin She had begun to gain weight rapidly four years earlier her face had become rounded and hypertension was then found She had been treated with digitalis and Novurit for 2 years She had been confined to bed for the last year because of repeated fractures of the ribs and fracture of the femoral neck She was given X ray irradiation 2 × 3 000 r to the hypophysis

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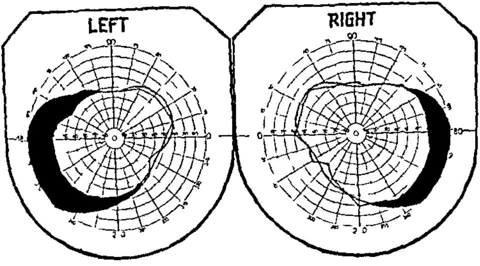


Fig 2 A Slight b emporal heteronym hem anops a (June 19 9

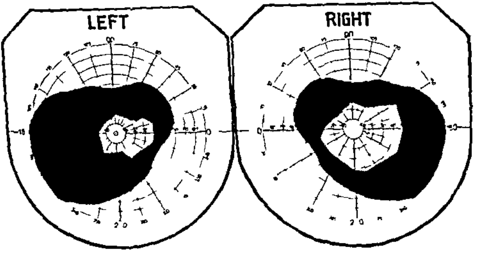


Fig B Severe t empor l he er n n hem n ps a partly oncen ri narrow ng Ap l 19 1

llow n c lx n he r gh a lrenal cortex The pan reas al ws a op h l po natos s and m a ns many small areas f necr s s The g n als a e a r p l ed There is severe s stem c n cop rns s

P h h ol g al hang H p p l s s Al r os le en re p ary s replaced by neop la c t e en losed n a caps le of fibrous connect e tiss e Normal p tu tary t ss e s found exclus ely in between the elements of the capsule in the f rm of a few compressed parenchymal cells Here we find alongs de the chro nophobe cells wh ch comprise the major ty a few eos noph l c and basoph l c cells too The neurohypo- ph s s cannot be recogn sed anywhere

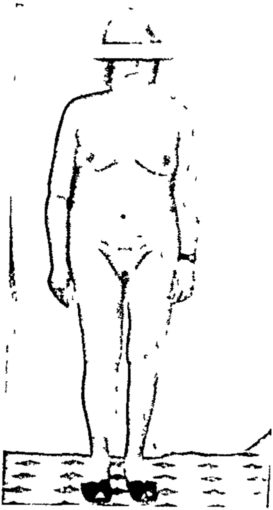


Fig 1 Typical Cushing s syndrome with hyperpigmented skin

with no substantial result She had been taking large doses of male and female sex hormones without benefit

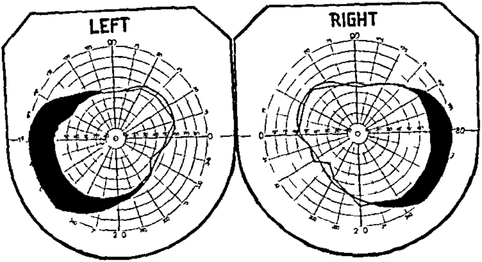
On admission moon face bull neck, adipose abdomen and relatively thin upper extremities were found There was generalised oedema The skin was hyperpigmented all over the body there were erythema of the face and ecchymoses of the lower legs (fig 1) Physical examination of the chest showed slight emphysema enlargement of the heart in both directions dull heart sounds accentuation of the second aortic sound Blood pressure 230/130 mm Hg pulse rate 96/min There was ascites in the abdomen the liver was enlarged extending 3 fingers breadth below the right costal arc

Laboratory findings ESR 2 mm/1 hour, RBC 4 0 million, WBC 5 200 Urinalysis specific gravity 1,022, albumin curdy precipitate, pus positive, ubg normal, sediment 2 to 3 WBCs Glucosuria around 30 g a day, with the patient on 40 units of insulin and 60 units of zinc protamine insulin NPN 30 mg % Endogenous creatinine clearance between 47 and 76 ml/min Liver functions tests negative Serum cholesterol 347 mg % Serum Na K, Cl, Ca and P values were normal 17 ketosteroids 20 2 mg in 24 hours ECG deviation of R to the left, serious disturbance of repolarization Fluoroscopy of chest normal lungs, enlargement of heart and dilatation of aorta Radiographs of vertebral column Porosis of cervical, thoracic and lumbar vertebrae, flattening of vertebral bodies *in thoracic area, diabolo-shaped third* lumbar vertebra (Dr G Laszka) Radiographs of sella turcica The sella is destroyed its usual shape is not discernible, only the anterior clinoid process can be visualized, the posterior processes being absent The area of sella is distended to about plum size The sphenoid sinus is not visible (Dr Jóna) Fundoscopic examination hypertensive and diabetic retinopathy, bilateral temporal decolorisation Examination of visual fields by perimetry in June 1959 bitemporal heteronym hemianopsia (Dr Walkowszki) in April 1961 bitemporal heteronym hemianopsia with concentric narrowing of field (Dr Eross) (fig 2) The patient died on the 27th of April, of heart failure

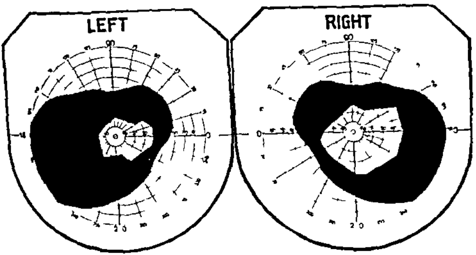
Clinical diagnosis Cushing s syndrome Intrasellar adenoma? Steroid diabetes Hypertensive decompensated heart disease Osteoporosis with pathological fractures Diabetic polyneuritis

Abstract from *autopsy* record The optic chiasma is elevated under it there is a nut sized neoplasm not adherent to the surrounding tissues filling out and bulging from the sella turcica but not rupturing the diaphragma sellae

The thyroid is slightly enlarged rich in colloid The adrenals are slightly enlarged the cortex showing only sulphur yellow spots There is a big bean sized node sulphur



I g 2 A Sl ght b en poral heter nym l emianops a June 19 9



I g ? B Severe bi empo l l eteronym hen an ps a p rtl on en r narrow ng Apr l 1961

yellow n col r in the r gl t a lrenal cortex Th pan reas sl ows atropl y l pc mat is s and c m a ns n any small areas of necros s Tl e gen als are atrop l ed There is severe system c os eop or sis

P h log al chang H pop h s s Al n w the en re p tary is repla ed b neopla c t ssue en l sed in a caps le of f bro s connective tissue Normal p tu tary t ssue is f u nd exclus ely in between the elen ents of the caps le n the f rm of a few con pressed p arenchymal cells Here we f nd alongs le tl e chromophobe cells wh ch comprise the major ty a few eosinoph l c an l basopl l c cells too The neurohypo p l sis cann t be recogn ed anywhere

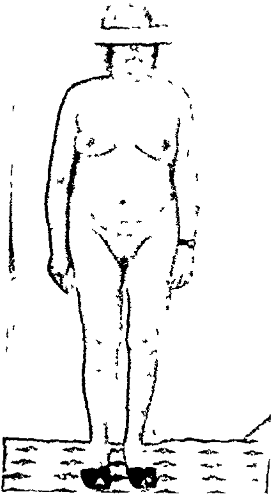


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Abstract from *autopsy* record The optic chiasma is elevated under it there is a nut sized neoplasm not adherent to the surrounding tissues filling out and bulging from the sella turcica but not rupturing the diaphragma sellae

The thyroid is slightly enlarged rich in colloid The adrenals are slightly enlarged, the cortex showing only sulphur yellow spots There is a big bean sized node sulphur

decisive proof, we reckon that on the basis of certain clinicopathological observations we may decide the issue

In the case of an 'active' adrenal cortical adenoma, atrophy of the contralateral adrenal would be expected As opposed to this, we have found hyperplasia of the adrenal cortex on both sides, by gross examination and by microscopic study alike (widened zona fasciculata) This suggests that the adrenal cortical adenoma was inactive, silent

The skin of the patient exhibited hyperpigmentation, as found also by others in Cushing s syndrome with chromophobe adenoma (1 11) And in a case of Cushing s syndrome with chromophobe adenocarcinoma the pigmentation was absolutely of the Addisonian type (5) Though it may appear to be paradoxical it is quite understandable pathophysiologically that in Addison s disease and in certain cases of Cushing s syndrome hyperpigmentation of the same type develops as a result of an overproduction of the melanocyte stimulating hormone parallel with the excessive ACTH secretion

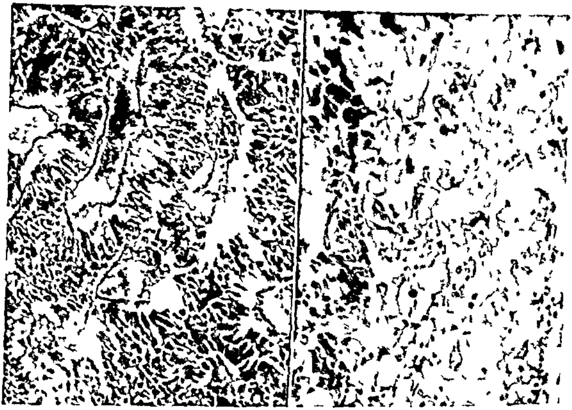
Thus there is very good reason to believe that in our case it was the chromophobe adenoma of the hypophysis that was endocrinologically active whereas the adenoma of the adrenal cortex was inactive

Danowski (3) has collected 20 cases *from the literature* in which adenomas occurred at the same time in several endocrine glands There was just one single case in which chromophobe adenomas and adrenal cortical adenoma were present together without giving rise to Cushing s syndrome On the other hand, in one of the cases of Cushing's syndrome described by Plotz et al (10) there were a pituitary adenoma of unclarified type (chromophobe?) and an adrenal cortical adenoma, but there was also atrophy of the contralateral adrenal cortex It seems therefore that in the latter case it was the adrenal cortical adenoma that was active and the pituitary adenoma that was inactive, just the opposite of what was found in our own patient

In agreement with other observations our case indicates that if 1 enlargement of the sella, 2 bitemporal hemianopsia and 3 hyperpigmentation are found, the possibility should be considered that the Cushing syndrome may be correlated with chromophobe adenoma or chromophobe adenocarcinoma As suggested by the poor results described in the literature in such cases the treatment of choice should not be adrenalectomy but irradiation and or surgery of the hypophysis In our case however the irradiation of the pituitary produced no convincing changes

It is conceivable that a case similar to ours may entail several pitfalls for the endocrine surgeon

1 Adrenalectomy is performed (on the side of the adenoma in the first *step)* virtually unilaterally no change may be anticipated
2 Bilateral adrenalectomy is performed the Cushing syndrome is cured but the tumour of the hypophysis expands
3 Hypophysectomy is *performed* no result ensues the pituitary tumour being silent and the adrenocortical



A B

Fig. 3. A. Characteristic part of hypophysial tumour. Accumulation of mostly cylinder shaped tumour cells with variable nuclear structure, neutrally staining fine cytoplasmic granulation around dilated vascular lumina. B. Accumulation of cells with puffy, foamy cytoplasm and small round nuclei in hypophysial tumour

The tumour itself is partly of glandular structure and is composed partly of groups of solid cells. The tumour cells are arranged at sites circularly or radially around dilated capillaries and are cuboid, cylindrical or polygonal in shape; the cytoplasm is pale and stains neutrally, showing fine granulation. No cytoplasmic granules resembling eosinophilic cells are visible in the neoplastic cells. The nuclei are mostly small, but a few big hyperchrome ones also occur; some nuclei show irregular shape and mitosis. In some areas the stroma is composed of hyaline connective tissue containing also precipitates of calcium. In some parts of the tumour groups of cells with big, puffy, foamy cytoplasm and small round nuclei also occur, resembling pseudo-xanthoma cells or eventually oncocytes. The latter may have been the products of degeneration (resulting from irradiation with X rays?). The pituitary tumour is a chromophobe adenoma (fig. 3 A and B).

Adrenals. The cortex is moderately dilated, the zona fasciculata is thickened. The cells of the zona reticularis are dissociated and vary in size; intercellular round cell infiltration is also visible in some areas. The tumour of the right adrenal cortex is composed of solid groups of polygonal cells with foamy cytoplasm (solid adenoma of the suprarenal gland).

Discussion

In our case the Cushing's syndrome could conceivably have been caused by both the pituitary adenoma and the adrenal cortical adenoma. Although there is no

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From the Department of Internal Medicine (Head Professor E Ask Upmark M D)
University Hospital Upsala Sweden

Systemic Scleroderma, Calcinosis Cutis and Parathyroid Hyperplasia

By

SVEN MÅRTEN SAMUELSSON and IVAR WERNER

Scleroderma occurs mainly in two different forms systemic and circumscribed scleroderma Acrosclerosis is the most common form of systemic scleroderma occurring in about 95 per cent of all cases (44 45) It usually affects women About 10 per cent of patients with systemic scleroderma also have calcinosis of the skin the so-called Thibierge Weissenbach *syndrome*

The nature of the metabolic disturbance behind the calcinosis is still obscure Many hypotheses have been suggested The tendency to calcification may primarily be due to the disease of the connective tissue Increased influence from or susceptibility to parathormone activity is another possibility Structural changes in the parathyroid glands and signs of impaired calcium metabolism have sometimes been observed

A case of acrosclerosis combined with calcinosis of the skin and parathyroid hyperplasia has recently been studied in the Department of Medicine, and is reported here as it seems to offer some interesting points for discussion

Case report

72 year-old 2 para woman who in 1927 had bilateral bronchopneumonia. In 1935 she noticed a small cutaneous induration of the left forearm From then onwards she noticed that her hands and feet easily became cold and cyanotic These symptoms gradually increased In 1940 there was a fracture of the right femoral neck. In 1949 a progressive cutaneous sclerosis affecting hands and feet was diagnosed Multiple telangiectasis appeared Numerous small hard tumours appeared in the skin near the small joints These tumours sometimes exulcerated leaving slowly healing scars They contained a chalk like mass which was shown to *consist of* calcium triphosphate crystals A few similar deposits were also found in *the* palms soles and lower parts of the legs

During the years 1952-62 she was admitted to the clinic and thoroughly examined several times X ray examination revealed widespread small interstitial calcifications in the legs Two small calcifications were also found in the tongue Considerable osteoporosis of

Submitted for publication December 7 [illegible]

adenoma active In such cases the new diagnostic methods (Metopiron test, Blood ACTH assay) will yield precise and reliable information (4)

Summary

Cushing's syndrome, chromophobe adenoma of the hypophysis and adenoma of the adrenal cortex occurred together in the same patient The adrenal cortex showed hyperplasia on both sides (there was no atrophy on the side contralateral to that with the adenoma), and the skin was conspicuously hyperpigmented On the basis of the clinical and pathological evidence it could be surmised that the chromophobe adenoma of the pituitary was endocrinologically active, whereas the adenoma of the adrenal cortex was inactive It is suggested that if there are 1 enlarged sella, 2 bitemporal heteronym hemianopsia and 3 hyperpigmentation, an association of Cushing's syndrome with chromophobe adenoma or adenocarcinoma should be suspected and therapy should be designed accordingly

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Urine			Comments
Phosphorus (mg/24 hrs)	Endogen creat. cl (ml/min)/ serum creat (mg %)	Phosph clear (ml/min)/ TRP (%)	
–			Ordinary diet
–			Ordinary diet
20			Ordinary diet + ACTH 40 IE × 5
		–	Ordinary diet
			Ca intake <200 mg/day
			Ca intake <200 mg/day + Versene locally
–			Ordinary diet
	60 1 1		Ca intake <200 mg/day
			Ca intake <200 mg/day + EDTA i v 3 g day
			One week after EDTA i v
	73 1 1		Ca intake <200 mg/day
			Ca intake <200 mg/day FDTA i v 3 g day
194	48 1 0	21 79	Ordinary diet
209	30 1		Ordinary diet
	1 6		Ordinary diet

Normal values after 19 9 serum calcium 8 4 10 4 mg % alkaline phosph 0 9 2 5 Bessey Lowry units

There were effusions within the pericardial pleural and peritoneal cavities The heart (weight 360 g was of firm consistency The right ventricle was hypertrophied The bases of the valves were calcified The lungs were firm There were signs of old inactive tuberculous lesions at both apical regions No remarkable changes were found in the gastrointestinal tract The liver was enlarged and firm The spleen was small and fibrotic The kidneys were somewhat small with a granular surface right kidney weighed 90 g left kidney 100 g The pituitary pancreas thyroid gland and adrenal glands were normal macro- and microscopically The ovaries were somewhat atrophic There were slight osteoporotic changes in the spinal column

The microscopical changes of the skin were pronounced There was extensive atrophy of the epidermis and thickening of the collagenous connective tissue of the corium The elastic fibres were fragmented The subcutaneous tumours of the hands and feet consisted of large cysts filled with calcific material There was marked atrophy of the skeletal muscles with interstitial fibrosis The myocardium was hypertrophied A pronounced interstitial pulmonary fibrosis was found The liver parenchyma showed marked changes associated with chronic venous congestion The renal parenchyma showed areas of glomerular hyaline degeneration and sclerosis of the arterioles

Three parathyroid glands were found One gland was of ordinary size the second was somewhat enlarged (8 4 × 4 mm) and the third distinctly enlarged (10 × 5 × 5 mm) The glands were greyish brown and of ordinary consistency Sections from the two enlarged glands showed areas of pronounced

TABLE I Blood levels and urinary excretion of calcium and phosphorus during the time of observation

Data	Blood			Urine
	Calcium (mg %)	Phosphorus (mg %)	Alk phosph units	Calcium (mg/24 hrs)
1949	9 8—10 3	3 7—4 1	—	—
1951	11 0	3 4	4 8	—
	—	—	—	147
1954	11 2	3 6	5 2	—
	10 6	—	—	31—69
	11 0	—	—	63
1955	10 9	3 5	6 5	—
1957	10 9—11 4	3 9—4 0	—	84—102
	8 6—9 8	2 2—2 6	—	*203—370*
	—	—	—	*215—225*
	10 9	—	—	36—77
	11 8	—	—	*278—347*
1961	10 0	3 2	—	80
1962				
Jan	9 8	3 1	2 0	56
March	10 8	3 0	2 8	—

Normal values before 1959 serum calcium 8—11 mg %, alkaline phosph 8—14 King Armstrong units

the right ankle was seen There were no apparent roentgenological signs of osteoporosis in the spine No calcifications were seen in the kidneys X ray examination of the gastro-intestinal tract was normal There was no steatorrhoea The heart had normal size and showed normal pulsations on X ray examinations the lungs had lowered borders and small inactive pleural indurations of the apical regions ECG showed sinus rhythm and unspecific changes of the ST T intervals

Repeated analyses of the serum concentrations and urinary outputs of calcium and phosphorus were made during different conditions The results are listed in table I The plasma cholesterol level was about 275 and total lipids were 900 mg/100 ml There were no haematological abnormalities The kidney function was normal except for the very last months

During the years many kinds of treatment were tried with no or doubtful effect vasodilating drugs, EDTA para aminobenzoic acid, ACTH, thyroid hormone quinethidine vitamin A physical therapy Repeated skin transplantations of the legs were made Some transient circulatory improvement was possibly seen after lumbar sympathectomy in 1951

Since 1954 she complained of angina pectoris increasing dyspnoea on effort and pitting oedema of the ankles In March 1962 she died at the age of 72 in cardiac decompensation gangraena of the right foot and uraemia 27 years after the appearance of the first symptoms of the disease

Autopsy

The immediate cause of death was pulmonary emboli dislodged from the femoral veins

TABLE II Earlier reports of increased calcium levels of the blood

Author(s) and year	Calcium (mg %)	Phosphorus (mg %)	Normal serum Ca accord to the author	Comments
Parkes Weber (1913)	Ca index 1 36	—	Ca index 0 9	One patient
Mosbacher (1919)	18 35 43 5	—	17 3 28 7	One pat Low calcium diet High calcium diet
Pulay (1921)	26 94 and 36 29	—	—	Two patients
Schamoff (1923)	0 43 5	—	—	One pat betw menstr per during menstr per
Weil et al (1924)	10 1—12 7	8 0—20 0	—	One patient
Mobdizanjanz (1925)	12 2 22 85	— —	— —	One pat betw menstr per during menstr per
Schultze (1925)	12	—	—	
Pernet (1926)	13 0			One patient
Weulman et al (1926	9 1 11 8	-	—	One patient Hypercholesterolaemia
Staub (1927)	16—17	5 5—9 4	12	One patient
Lortat Jacob et al (1929)	0 28 ‰	—	0 12 ‰	One patient
Naegeli (1929	13 –38	4 5 5 1	10—12	6 of 20 patients Decrease of serum Ca after treatm with Collip s parathormone
Pautr[illegible] et al 1929	10 9[illegible] 12 28	—	8 9 9 9	Six patients
Hillman 1930)	90	—		One patient
Lewy 1930	11 9[illegible] 12 28		10 3	One patient
M[illegible]an et al 1930 and M[illegible] 1931)	11 8			One patient Parathyroid adenoma
Weissenbach et al 1931	12 [illegible]	13 1	9 5 11 0	Ionized calcium normal hyperch lesterolaemia
Skoog [illegible] 1932	13 1 14 9			Serum Ca normalized after ingestion of ammonium chlor
J[illegible]ng [illegible] al 1932	10 0 11 [illegible]		P > 10 0	12 of 15 patients
H[illegible] 1933	[illegible]		—	A few months lat r n rmaliz
W[illegible]enbach at al 1933	1[illegible] 1 12 8 11 12 5	—	9 [illegible]—11 0 9 5 11 0	One patient One patient
Donati 1933	Increased		—	One patient



A B

Fig 1 Photomicrographs of one of the enlarged parathyroid glands (courtesy of Drs Nathorst Windahl and Olding) (van Gieson × 150) A areas of hyperplasia of chief or (partly) oxyphil cells B areas of water clear cells

hyperplasia of oxyphil or chief cells with partly follicular arrangement Here and there water clear cells were seen The parathyroid hyperplasia was histologically of the secondary type (fig 1)

Case summary

Typical history of slowly progressive generalized scleroderma complicated by severe calcinosis cutis The laboratory findings showed a serum calcium level around and sometimes above the upper normal limit The serum phosporus and the urinary calcium output were usually normal The renal phosphate clearance was elevated At autopsy hypertrophied parathyroid glands were found The chemical and histological findings suggested the presence of a mild secondary hyperparathyroidism

Discussion

This case of scleroderma is interesting with regard to the prominent calcinosis of the skin and the parathyroid hyperplasia found at autopsy

Few metabolic studies of calcium and phosphorus have been made in patients with scleroderma The parathyroid glands seem to have been examined histologically only occasionally in such patients Most authors have found normal values of calcium and phosporus in blood and urine (37, 40) In a clinical study of 727 cases of systemic scleroderma, determinations of serum calcium in 173 patients gave normal results in all but one patient having coexistent hyperparathyroidism" (44, 45) No details of this patient were given Normal values of serum phosphorus were found in 87 of the patients Some patients with scleroderma have, however, been reported to have slight or pronounced hypercalcaemia Some of these values are very difficult to evaluate in view of the unreliability of the laboratory methods used In another study on 65 patients with scleroderma, hypercalcaemia of 10—12 6 mg % was found in 48 cases (28) The normal range was considered to be 8 5—10 0 mg % In a study of 614 earlier published cases

TABLE II Ea l r reports of ncreased calcium lev ls of the blood

A hor s) and y ar	Calc um (mg %)	Phos- phorus (mg %)	Normal serum Ca accord to th au hor	Comments
Pa kes W ber (1913)	Ca ndex 1 36	—	Ca index 0 9	One patient
Mosba h r (1919	18 35 43 5	—	17 3 28 7	One pat Low calcium d et H gh calc um d et
Pula (19 1)	26 94 and 36 29	—	—	Two pa ents
Scha noff (19 3)	0 43 5	—		One pa betw menstr per dur ng menstr per
W et al. 19 4	10 1—12 7	8 0 20 0	—	One pa ent
Akobdzan anz 1925	12 2 22 85	— —	—	On pa be w mens r per dur ng men tr per
Schul ze 19 5	12			
Pem (1926	13 0			One pa ent
V ssdman e al 1926	9 1 11 8			One pa nt H pe choles e olaem a
S aub 19 7	16—17	5 5—9 4	12	One pa en
Lorta Ja ob et al 19 9	0 28		0 12	One pa ent
Naeg 19 9	13 38	4 5—5 1	10 12	6 of 20 pa en s Decrea e of erum Ca af er trea m w h Col p s para hormone
Pa al 1929	10 92 12 28		8 9 9 9	S x pa ents
H l an 1930	00			One pa ent
Lewy 1930	1 9 2 8		10 3	One pa ent
M an al 1930 an l M nlong 1931	1 8			One pa en Para hyro d ad noma
W we ba h al 1931	1 5	3 1	9 5 11 0	Ion zed cal um normal hypercholes olaemia
Sk weg renko 93	13 6 14 9			Serum Ca normal zed af r nges on of ammon um hlor
Ju g a	10 0 11		8 10 0	12 of 15 pa n s
H 1933	1			V f w mon hs la r normal z
V wr bach a al 93	12 1 1 8 1 1 5	—	9 5—11 0 9 5—11 0	On pa ent On pa ent
D e 933	In reased			One pa ent

Table II Cont

Author(s) and year	Calcium (mg %)	Phosphorus (mg %)	Normal serum Ca accord to the author	Comments
Billet et al (1933)	12 0	—	*Method of Guillaumin*	*After ligature of art thyr inf* serum Ca 10 5
Alessandri (1933)	11 0	2 17	Meth of Kramer Tisdall	After ligature of art thyr inf serum Ca 9 0
	11 5	—	Meth of Kramer Tisdall	After parathyroidect serum Ca 9 4
Santi et al (1933)	10 8	—	—	After ligature of art thyr inf serum Ca 10 2
Brooks (1934)	11 3—13 7	5 63—8 62	Meth of Kramer Tisdall	Serum Ca phosph and alkal phosphat normalized after ingestion of alkaline sodium phosphate
Basch et al (1934)	10 1—10 9	11 0	9 0—10 0	After parathyroidect and ligature of art thyr inf serum Ca 10 1
Laurent (1934)	11 5	—	—	After ligature of art thyr inf serum Ca 8 5
Payrau (1934)	10 9 – 12 4 – 12 6	—	8 5—10 0	Cases No 20 25 and 47 from the thesis (not reported elsewhere)
Costello (1935)	17 5—14 2	3 38—3 78	9—11	Serum calcium normalized three years later
Leriche et al (1931—1937)	10 0—11 [illegible]	—	8 5—9 9	13 of 20 patients Serum calcium normalized after parathyroidectomy in 5 cases
Carcassonne (1937)	12 0—12 [illegible]	—	8 5—10	After parathyroidect and ligature of art thyr inf serum calcium 10 0
Ochsner et al (1939)	Increased	—	—	One pat After parathyroidect serum Ca normalized
Ramsdell (1939)	11 7	3 38	—	One patient
Fontaine et al (1946)	10 8	low	9 0—10 0	One patient
Tanret (1946)	11 8	3 6	—	Two parathyroid glands with abundant chromophobe cells
Teutschlaender (1911 1949)	11 36—19 9	—	—	Lipoido calcinosis One para thyr gland hypertroph and ossified Hypercholesterolaem

Table II Cont

Author(s) and year	Calcium (mg %)	Phosphorus (mg %)	Normal serum Ca accord to the author	Comments
Mallet-Guy et al (1950)	12.2	—	<10.5	Parathyroidect Ca afterwards 9.0
	12.4	—	—	Parathyroidect. Ca afterwards 10.4
Halper (1952)	12.3	4.2	—	One patient
Annamunthodo (1960)	10.1—12.6	7.8—8.1	—	One patient

of progressive scleroderma, hypercalcaemia was found in slightly less than 10 per cent (30). The authors however, did not give any details. In contrast to these findings a few cases of calcinosis have been reported to have hypocalcaemia or signs of hypoparathyroidism (5 28). Cases with hypercalcaemia found in the literature are listed in table II.

Balance studies on calcium and phosphorus are difficult to perform and evaluate. A tendency to calcium and phosphorus retention in the body was found in careful determinations over long periods by Cornbleet and Struck (8). A similar tendency has also been found in short time studies (e g 3 6). A positive *calcium balance* and a tendency towards a negative phosphorus balance was *found during* a 2 month study by Byron and Michalover (7). In another study *calcium balance* was in equilibrium in four cases, positive in one and negative in four (2). Salvesen and Boe found a normal metabolism of calcium and phosphorus on an ordinary diet and a negative balance on a low calcium diet in two patients with scleroderma. This was considered to be a normal response (35). On a low calcium diet alone or in combination with a ketogenic diet calcium balance was slightly negative in one patient (1). Negative calcium balance was also found by Goetz (11) and Weissman (47).

Serum calcium was not restored to normal three hours after injection of calcium chloride in some patients with scleroderma (34). Other calcium tolerance tests were also abnormal in these patients (4). The injection of parathormone in one patient with scleroderma gave only a slight increase in serum calcium whereas there was a marked response in a control (15).

The parathyroid glands have been examined in patients with scleroderma either after removal of the glands in an effort to cure the disease or at autopsy. Examination of all four glands is however lacking in almost every case. Kraus examined three parathyroid glands in a case of systemic scleroderma and periarteritis nodosa (14). Two glands were distinctly enlarged. They showed a histological picture of chronical, interstitial parathyroiditis. Amyloid infiltration of the parathyroids was de

scribed in a patient with generalized calcinosis and amyloidosis without tetany (38). Serum calcium and phosphorus varied from normal to tetanic values. In a severe case of scleroderma and osteoporosis reported by Bauer, autopsy revealed an enlarged parathyroid gland with histological signs of hyperplasia (3). No values were reported for serum calcium. In another case of scleroderma reported by Milian et al. (24) one parathyroid gland was removed. The histological picture showed an adenoma with chief cells. The serum calcium was 11.8 mg %. In a series of 10 patients with scleroderma treated by parathyroidectomy Leriche et al. found elevated serum calcium values in five (21). In all cases the calcium values became normal postoperatively. No report of the histological picture of the glands was given. In other reports they described one case with histological signs of parathyroid hyperfunction and two cases with greatly increased vascularization of the glands. The serum calcium level in these patients was elevated and returned to normal after the removal of the glands (for ref., see (20)). Similar findings have also been reported by others (22, 27, 32, 42). Bernheim and Garlock performed parathyroidectomy in 17 cases of Raynaud's disease and scleroderma (4). Two or three glands were removed in each case. In seven of these cases the glands were enlarged and showed hyperplasia. In four cases the glands were enlarged with a normal histological picture. In two other cases the glands were enlarged but no histological examination was made. Two cases showed a picture of parathyroiditis and one case had microscopical abscesses. Gould et al. found a case of wide spread calcinosis, who was shown to have an enlarged parathyroid gland with atrophy and fatty infiltration at the autopsy (12). A chromophobe adenoma of the pituitary, nephrolithiasis and pyelonephritis were also found.

Teutschlaender reported a few cases of a disease called lipoido calcinosis (43). One case had widespread calcinosis, hypercalcaemia and pronounced osteoporosis. The patient died in uraemia. At the autopsy one parathyroid gland was found to be hypertrophied and even partly ossified. According to the author the histopathological picture with hyperplasia of the chief cells was in favour of primary, not secondary, hyperparathyroidism in spite of the findings of small, uraemic kidneys. In the majority of cases with scleroderma who have had their parathyroids removed the histological picture was normal (7, 20, 22, 26, 46). Yet some of these patients had hypercalcaemia, which was normalized after removal of the parathyroid glands (22, 26, 46).

Thus chemical and histological changes suggesting mild hyperparathyroidism do not seem to be very uncommon in scleroderma. A clinically prominent hyperparathyroidism seems, however, to be very rare.

Soft tissue calcification may occur in hyperparathyroidism, usually in cases with impaired renal function. The most common sites of the calcium deposits are the kidneys, pancreas and the periarticular tissues. A few cases with widespread calcifications of the cardio

vascular system, the lungs, the muscles, the gastro-intestinal tract and the subcutaneous tissue have also been reported (37) Scleroderma like lesions have been observed in two cases of clinically apparent primary hyperparathyroidism (13, 17) No case with calcinosis cutis and primary hyperparathyroidism seems to have been reported

During certain experimental conditions injection of parathyroid hormone in the rat is known to produce cutaneous calcinosis and sclerosis resembling the calcifying type of scleroderma (20 37)

It has also been shown that similar lesions can be produced in rats treated with vitamin D preparations, for instance dihydrotachysterol (37) Prolonged ingestion of vitamin D may cause circumscribed calcifications also in man (10)

Some experiments have spoken in favour of a deficient diet being the cause of the calcinosis Thus by excluding a fat soluble antistiffness factor', normally found in greens a similar disease has been produced in guinea pigs (16) Patients with calcinosis treated with this factor were not improved however (16) Similar lesions have also been produced in animals given acid diets high in calcium and phosphorus or diets high in phosphorus and ergosterol

The uncommon form of scleroderma called lipoido-calcinosis reported by Teutschlaender and others was considered to be the result of a defective lipid metabolism giving rise to a general disease of the locomotor system 43 Hypercholesterolaemia always present in lipoido-calcinosis is usually not found in ordinary scleroderma Other evidence of an impaired lipid metabolism is usually also lacking

A deranged interaction of the serotonin monoamine-oxidase mechanisms at the tissue level was postulated by Zarafonetis in a case of functioning carcinoid associated with scleroderma (48) Abnormal tryptophan metabolism was observed in a few cases of scleroderma by Price et al (31) The telangiectasis often seen in scleroderma might be connected with a high serotonin level

Scleroderma may sometimes be associated with disturbances from endocrine glands other than the parathyroids (37) Thus it may be associated with acromegaly, pituitary haemorrhage and inflammation hypo- or hyperthyroidism, lesions of the pancreas Addison's disease adrenal neoplasm and ovarian dysfunction However there is no evidence that scleroderma may be caused by any of these endocrine disorders

An association of cancer of the lung and scleroderma has been noted (29) In this connection it may be pointed out that bronchogenic carcinoma and some other neoplasms may cause *hyperparathyroidism* like syndromes (36)

From many experiments in animals and from clinical observations Selye has tried to find a common aetiology of calcinosis and some other disorders of the connective tissue He has created the concept of calciphylaxis (37) This phenomenon is a condition of induced systemic hypersensitivity developing after treatment with a sensitizer, for instance parathormone vitamin D, vitamin D_2 or dihydrotachysterol After a critical period an injection of a

"challenger", for instance ferric-dextran, chromic chloride or albumin, may cause a local calcification at the site of administration. Injection of other "challengers", for instance histamine liberators, such as 48/80, polymyxin or glucocorticoids may cause systemic calcification not at the site of application but in tissues for which these compounds seem to have particular affinity.

Many seemingly divergent observations are explained by this theory. Thus the "sensitization" of some scleroderma patients, resulting in calcinosis, might be caused by hyperparathyroidism. The activation of the parathyroids might for instance be caused by factors in the diet, malabsorption (19, 39) or renal insufficiency. It is possible that primary hyperparathyroidism in special conditions might produce cutaneous calcinosis under the influence of different "challengers". Possible challengers might develop from changes in the metabolism of metals such as iron, copper or zinc or from changes in connective tissue mucopolysaccharides. From the experiments of Lehr it is also known that disseminated cardiovascular and smooth muscle necrosis similar to the picture in scleroderma may be elicited by overproduction of parathyroid hormone in uraemic rats (18).

Equally plausible is, however, the assumption that the connective tissue lesion *per se* sometimes may cause a tendency to the deposition of calcium salts. The continuous trapping of calcium may stimulate the parathyroids to increased production of hormone, resulting in a state of secondary hyperparathyroidism. The findings of "secondary" hyperplasia in earlier reports and in our case are readily explained by this theory. When this hyperplasia has occurred, the parathyroid glands may in some cases continue to produce an excess of hormone, with further aggravation of the sclerodermatous lesion.

In many cases of scleroderma with calcinosis parathyroidectomy has been performed. Some of these cases have been improved for a longer or shorter time (20), but relapses are the rule (4). These cases may have improved chemically but the nature or course of the disease itself is not altered. This also fits in very well with the assumption that the hyperparathyroidism is secondary to the connective tissue disease.

Other methods of treatment have been tried, for instance the infusion of sodium EDTA (23, 25, 31, 41), ketogenic diet, ingestion of ammonium chloride (35), alkalizing compounds, for instance sodium citrate or phosphate (6, 33), thioguanine (9), all with questionable or variable effects. In our case EDTA increased the urinary output of calcium but there was no clinical improvement. Probably most of the urinary calcium arose from the osseous system and not from the calcium deposits of the subcutaneous lesions. Treatment with adrenal cortical hormones, which has been suggested, is probably dangerous, at least in cases with renal insufficiency, as judged by experiments in animals where corticosteroids have a powerful potentiating effect upon muscular necrosis elicited by overproduction of parathormone (18, 37). Different operations upon the sympathetic nerve system have sometimes effected considerable symp-

tomatic relief (20, 21), whereas agents causing peripheral vasodilation are of minor importance Many other agents, not mentioned here, have been tried without effect

Summary

A case of systemic scleroderma with severe calcinosis and histological signs of hyperparathyroidism is reported

The literature on similar cases with parathyroid engagement and disturbances of the calcium metabolism is reviewed and the possible mechanisms are discussed The clinical, chemical or histological signs of hyperparathyroidism sometimes observed in these cases are most probably secondary to the primary disease of the connective tissue

No method of treatment so far tried seems to have effected clinical improvement of any importance Corticosteroid therapy, often uncritically used in all types of collagen disease' may be especially dangerous in these cases

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Reversible Myopathy in Chloroquine Treatment

By

S Blom and P O Lundberg

Increasing attention has been paid in recent years to myopathies and peripheral neuropathies as symptoms in general diseases of a primarily non neurological nature The neurological symptoms frequently occurring in diabetes, thyrotoxicosis, Cushing s syndrome porphyria ethylism etc can be mentioned by way of example It is also well known that certain pharmaceutical products, such as corticosteroids thalidomide, isoniazid and nitrofurantoin, can present similar pictures especially in long term treatment

The antimalarial chloroquine phosphate has come into frequent use for collagenoses during the last ten years It is well known that for instance visual disorders secondary to changes in the cornea and the retina not infrequently arise with this therapy There are also some reports of muscular weakness that has been interpreted as neuropathy or neuromyopathy and which have been ascribed to chloroquine treatment (3 4)

At the Department of Neurology in Uppsala we have observed a case of reversible muscular weakness with relatively alarming symptoms which is in all probability ascribable to chloroquine treatment

Submitted for publication December 9 1964

Case report

The patient is a woman born 1931 *unipara* Family history of allergy and joint disorders No hereditary myopathy or other neurological disease The patient has suffered from slight asthma hay fever and eczema since childhood *In June 1961* pains first appeared in hand and foot joints with swelling of the latter ESR 30 mm/1 h Treatment 250 mg chloroquine daily *August 1961* untroubled by previous complaints but in *September* the joint pains returned and the chloroquine dosage was accordingly increased to 500 mg daily

In November 1961 she became aware of weakness in her legs had difficulty in going up and down stairs doing the housework and moving quickly On one occasion she fell headlong to the floor As a result she was examined at the end of November 1961 and paresis of the quadriceps muscles was noted She could not raise her legs more than 45 in the supine position

EMG I (29/11/1961) Normal insertion activity in all the muscles examined No fibrillations or positive waves Patches with

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proximal muscles of the leg This progressed during two months' continued treatment and about fourteen days after chloroquine had been withdrawn Neurologically, an atrophy and paresis were found, particularly of the thigh muscles, as well as abolished knee and ankle jerks Electromyographically and microscopically, there were changes resembling a myopathy The patient gradually improved during the year following withdrawal of chloroquine and is at present, three years after onset, completely restored to health

Discussion

This case conforms in all essentials to the four cases described by Loftus (3) and the four + three cases reported by Whisnant et al (4) At the Scandinavian Congress of Neurology in Gothenburg 1964 Blomberg (1) mentioned a case of dystrophia myotonica probably caused by chloroquine As early as 1951 however Lane (2) and later also others, noticed weakness in the leg as a side effect in patients treated with chloroquine and other antimalarials for amoebiasis

There is sufficient data from which to form a definite clinical picture The symptoms first appear after treatment with chloroquine in daily doses of 250—500 mg during 6 months — one year The symptoms then progress rather quickly and also appear to do so in the weeks immediately after withdrawal of the preparation (accumulation in the liver?) The patient then improves slowly and may be fully recovered after some months to a year The first clinical symptom is usually difficulty in walking up or down stairs Neurological examination reveals atrophy and paresis of proximal muscles, particularly in the thigh The knee and ankle jerks are nearly always abolished and the arm reflexes too, can be diminished Sensory symptoms are nearly always lacking and neither are there symptoms from the central nervous system

Clinically, the syndrome thus closely resembles progressive muscular dystrophy of the limb girdle type Electromyographic and microscopic investigations were made in four of the cases published by Whisnant et al as well as in our own However, the changes vary from case to case It is clear that the picture, in the first place, indicates a myopathy Certain data e g the loss of motor units (also noted by us) and the rather low speed of motor conduction in one of the cases of Whisnant et al indicate that there may also be a neurogenic injury Another noteworthy feature is the abolished reflexes even when atrophy and paresis are not very pronounced Nothing however indicates that a more extensive injury to the peripheral nerve can arise Whisnant et al have called the syndrome a neuromyopathy In our opinion the damage is primarily in the musculature, but the small nerve end plates in the muscles can also be affected Histological investigations to support this however are still lacking

In our experience the clinical picture also recalls the so-called steroid myopathy It is clearly distinguished from nerve injury caused by thalidomide nitrofurantoin or isoniazid In most of the cases previously described the patients have

numerous polycyclic potentials were found, especially in the thigh musculature (vastus medialis and rectus femoris bilat) and an *increased number of potentials* of abnormally short duration for these muscles The interference picture on attempted maximal contraction was sparser than normal in the vastus medialis muscle, indicating a loss of motor-units

Admitted to the neurological clinic 15/12/1961, by which time her condition had further deteriorated She could no longer go out of doors, could not walk up or down a staircase without banisters, experienced certain difficulties on swallowing and had also noticed a reduction in the girth of her thighs She had lost 2 kg in weight On examination, slight eczematous changes were found over the knuckles (atopic eczema) No joint changes General condition otherwise normal *Neurologically* muscular atrophy in both thighs, considerably impaired strength on flexion at the hip joints, Unable to climb up onto a chair The sternocleidomastoid muscles some what weak bilaterally Normal sensibility The knee and ankle jerks were abolished, the other tendon reflexes diminished No myotonic reaction *Sample biopsy I (16/12/1961)* from the vastus medialis muscle muscle fibres of ordinary but somewhat varying calibre, possibly some increase in the number of nuclei at the periphery of the fibres, otherwise normal *Chloroquine withdrawn 28/12/1961* At the beginning of January 1962 her condition had if anything, somewhat deteriorated, with increased weakness in the posterior muscles of the thigh *EMG 2 (3/1/1962)* Slight progress with a further loss of motor units particularly in the proximal leg muscles As before the remaining motor unit potentials resembled the picture in myopathy *Sampl biopsy II (9/1/1962)* from the biceps femoris muscle marked variations in calibre with considerable increase of nuclei at the periphery and interstitially, and proliferation of interstitial fat tissue The transverse striation was largely retained Still no inflammatory changes The patient was discharged from the hospital on 31/1/1962 No therapy

At a follow up in April 1962 the patient reported an improvement She could now walk freely on the level, but still had some difficulty in going up and down stairs It was found that she could climb up onto a chair Both knee jerks were now elicitable, as was the ankle jerk on the left side The right ankle jerk was still abolished

In May 1962 her condition was subjectively and objectively as before *EMG 3 (7/5/1962)* unchanged picture, possibly somewhat denser interference in the vastus medialis muscle on maximal contraction Speed of conduction in left peroneal nerve 45 m/sec (normal value)

September 1962, further subjective improvement she walked up and down stairs without trouble and took part in gymnastics for housewives Reported that spring board exercises were somewhat tiring *EMG 4 (24/9/1962)* only slight signs of myopathy in the thigh muscles, i e a somewhat increased number of polycyclical action potentials but no loss of motor units

In September 1964 the patient was reported to have no complaints either in respect of her joints or muscle power She had already had another child and was again pregnant

Other examinations during hospitalization in 1961 ESR 30—9 mm/1 h Serum electrophoresis no specific changes WBC 8 200—5,900/mm³ Differential count 25—10 % eosinophils On one occasion partial atypical lymphocytes Total lipids 1,200 mg % Cholesterol 386 mg % PBI 7 0 μg % BMR + 1 % Red cell uptake of triodothyronine 17 1 % No LE-cells found CRPA neg Sensitized sheep red blood cells and acryl test neg AST 280 units/ml ASTa 1 25 units/ml Meinicke Kline, Wasserman neg Creatine in urine not detectable Creatinine in urine 1 155 mg/24 hours No porphobilinogen or coproporphyrin III found in urine X ray of lungs cranium pelvis and hip joints normal

Summing up it can be said that the patient after five months' treatment with 250—500 mg chloroquine phosphate daily, began to notice a paresis of the

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Vitamin B_{12} Hypovitaminosis in Mental Diseases

By

E. Edwin, K. Holten, K. R. Norum, A. Schrumpf and O. E. Skaug

The occurrence of mental symptoms in connection with pernicious anemia has been known for years (6, 11, 12, 17, 27). Addison in his classical description wrote of the patient's mind "as occasionally wandering" (2). Eilenberg (10) in 1960 reported 20 patients with psychiatric illness in pernicious anemia. Only 4 of his patients improved on B_{12} therapy. It must be stressed that mental symptoms may appear years before anemia develops (27) and no strict relationship seems to exist between anemia, if present, and the degree of lesion in the central nervous system (32). Adams and Kubik (1) showed that the pathological process in the central nervous system involves the white matter as a more or less diffuse scattered degeneration. Treatment with vitamin B_{12} may completely reverse the degenerative changes if recognized in time. The pathogenesis is not clearly understood.

Scheinberger (25) found a good correlation between severe neurological involvement, abnormal mental status and low cerebral oxygen consumption. The cerebral metabolism normalized to some extent during vitamin B_{12} therapy.

It has recently been suggested that the methylmalonic acid accumulating in B_{12} deficiency might be toxic and give rise to some or even all of the symptoms in pernicious anemia. This metabolic lesion is due to a breakdown of the cobamid (B_{12} coenzyme) governing isomerization of methylmalonyl Coenzyme A to succinyl Coenzyme A. Methylmalonate accumulates and is excreted in the urine from patients with pernicious anemia. The metabolic defect is corrected by vitamin B_{12} treatment. The neurological symptoms of pernicious anemia are well known (16, 21, 22). EEG may be abnormal (30). Mental disturbance is quite common and may be the first symptom. No distinct psychiatric syndrome has so far been reported. The symptoms are extremely variable and may range from mild mood disorders to

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had corticosteroids or antimalarials apart from chloroquine This makes the causal connection in these cases somewhat less certain The primary disease can of course also play a part All the cases published by Loftus and Whisnant et al were treated because of collagenosis It is well known that this group of diseases also attacks the musculature Investigation by muscle biopsies in the cases examined, however, gave no support for specific changes In our case, the cause for chloroquine treatment was rather dubious Neither the course of the disease nor any serological tests indicated that there was in fact a collagenosis in this case Neither had the patient received any medicine other than salicylates, over and above the chloroquine Our case can therefore be considered to be pure"

A strange circumstance is that of the thirteen cases described, no less than eleven are women This, however, can conceivably be because the diseases indicating the treatment are more common in this sex

It is clear that antimalarials are certainly very effective, but also extraordinarily toxic Possible metabolic explanations for this will not be gone into here There are also a great many animal experiments illustrating the toxic effect of chloroquine and other antimalarials on different tissues, including the musculature It is important to bear this in mind and to be on the alert, so that the preparation is withdrawn in time should any side-effects appear One cannot assume that the pareses and atrophies are fully reversible after prolonged medication with chloroquine, particularly if, as seems possible, a neuropathy can also arise

Summary

A case is described of pronounced muscular weakness, particularly in the thigh, and loss of reflexes in a woman treated with chloroquine The changes are interpreted primarily as a myopathy The patient's symptoms disappeared in this case after the preparation had been withdrawn

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rich in liver together with hydrochloric acid

Rafaelsen and Schou (23) investigated the serum vitamin B_{12} level in 41 psychiatric patients above 30 years and with achylia gastrica In 10 patients they found a serum value below 150 pg/ml and in 4 below 100 In 111 demented patients above 60 years and without screening according to the gastric acid secretion they found 20 below 150 pg/ml and 9 below 100 In 142 old patients without dementia from various hospitals they found 11 below 150 pg/ml and 1 below 100 All pathological values had been confirmed by at least 2 analyses Droller and Dosset (9) found significantly lower serum vitamin B_{12} values in senile demented patients when compared with normals of the same age

The aim of the present study was to investigate the frequency of vitamin B_{12} deficiency among patients admitted to a mental hospital and further to ascertain whether any particular patients presented symptoms which might indicate a B_{12} deficiency

Material and methods

The material consists of 199 male and 330 female patients admitted to Lier Mental Hospital during the period of Nov 1 1961 to Oct 31 1962 Of the male patients 86 patients were admitted for the first time and 113 were readmitted Of the female patients 148 were admitted for the first time and 182 readmitted As a vitamin B_{12} deficiency is more likely to appear in patients above 30 years of age no patients below this age were included The present material therefore consists of 396 patients 256 female and 140 male patients As will be shown later a few cases with a low serum vitamin B_{12} level were found in young patients hence the limitation to patients above 30 years of age was not fully justified

In all patients a complete medical, psychiatric and neurological examination was carried out In addition to the clinical examination the EEG was recorded A blood smear was made from all patients and assessed by three examiners independently The ESR was estimated and also the hemoglobin value and serum vitamin B_{12} level In all patients with serum vitamin B_{12} levels below 100 pg/ml supplementary examinations were made red blood cell count mean corpuscular hemoglobin serum iron fractionated examination of gastric juice after histamine stimulation Schilling test and bone marrow examination

Blood examinations and serum iron determination were carried out according to accepted methods used in most laboratories Schilling tests (26) were performed with radiocyanocobolamin obtained from Abbott Laboratories and according to the manufacturer s recommendation

A Schilling test of 7 % was considered as the lower normal limit It has been shown by Persson and Hansen (20) that morphological bone marrow lesions characteristic of vitamin B_{12} deficiency usually accompanied a Schilling test below 5 % Vitamin B_{12} determinations were made by the Euglena Gracilis method (24)

The reproducibility of the method was investigated in the following way From 3 different pools of normal serum aliquots were transferred to test tubes and kept frozen at −22 C Aliquots were selected at random during a two-month period and analysed for vitamin B_{12} content From pools I and III dilutions were made with 0 9 NaCl solution to a final serum concentration of 50 and 60 % for I and 50 and 70% for III This was done in order to check the method within the low ranges of vitamin B_{12} concentrations

As will be seen from table I the reproducibility of the method is fairly good The

TABLE I Reproducibility of the method for vitamin B_{12} determination Figures in brackets Values found by B_{12} determination, followed by the value corrected for the dilution

	Sample no (pg/ml)					Dilution			
	1	2	3	4	5	50%	60%	Mean	SD
Pool I	170	156	150	185	—	(85) 170	(109) 182	169	13 8
Pool II	213	228	364	318	374			229	69 3
						50%	70%		
Pool III	239	276	297	288	—	(130) 260	(225) 322	280	26 5

TABLE II Serum vitamin B_{12} levels in the material Figures in brackets % of the total number investigated

	No	Pathological values (0–100 pg/ml)	Subnormal values (101–150 pg/ml)	Path + subnorm values (0–150 pg/ml)	Normal values (above 150 pg/ml)
Female	256	17 (6 5%)	28 (10 5%)	45 (17%)	211 (83%)
Male	140	6 (4 0%)	10 (7 0%)	16 (11%)	124 (89%)
Total	396	23 (5 8%)	38 (9 6%)	61 (15 4%)	335 (84 6%)

TABLE III The frequency of serum vitamin B_{12} values below 100 pg/ml in various normal materials The Euglena gracilis method has been used by all authors cited except Rafaelsen and Schou who used Lactobacillus Leichmannii

Author	No examined	Age (yrs)	Serum vitamin B_{12} (pg/ml) 0—100	Range
Mollin & Ross (17)	123	15—93	3	60— 720
Lear & al (14)	95	—	0	115—1 330
Killander (12)	56	—	0	120— 710
Droller & Dosset (8)	300	19—90	0	110—1 005
Rafaelsen & Schou (22)	142	above 60	1	—
Total	716		4	

violent maniacal states, stuporous depression and overt visual and auditory hallucinations

The frequency of vitamin B_{12} deficiency in a general psychiatric material has not been investigated to any extent In 1928 Warburg and Jörgensen (31) pointed out the connection between achylia gastrica and psychiatric illness Most of their patients improved on a diet

TABLE IV The average of vitamin B_{12} values in 10-year age groups

Female				Male		
Ag	B_{12} (pg/ml)	S D	No	B_{12} (pg/ml)	S D	No
30–39	298	132	42	306	154	29
40–49	360	144	58	329	179	40
50–59	262	111	65	269	110	33
60–69	310	166	47	266	178	31
70–79	184	79	35	214	71	6
80–	205	135	9	520	–	1

TABLE V Hemoglobin compared with serum vitamin B_{12}

	No	Serum vitamin B_{12} (pg/ml)	Hemoglobin (g/100 ml)	S D	t	p
Female	211	150	13 7	1 47	4 018	< 0 001
	45	0 150	12 8	1 34		
Male	124	> 150	15 4	1 58	2 817	0 02 > p > 0 01
	16	0–150	13 8	2 20		

TABLE VI The frequency of anisocytosis and hypersegmentation of granulocytes in peripheral blood together with vitamin B levels

	No	Serum vitamin B_{12} (pg/ml)	Anisocytosis found in No	%	Hypersegmentation No	%
Female	17	0–100	6	35	5	29 5
	45	0–150	14	31	9	20
	211	150	19	9	12	6
Male	6	0–100	0	0	1	17
	16	0–150	4	25	5	31
	124	> 150	10	8	8	6 5

tion (28) In such cases an increased sedimentation rate would be expected

Peripheral blood picture

A blood smear was made from every patient In one single case the smear indicated pernicious anemia In this case the disease had been known for years

As will be seen from table VI, anisocytosis and hypersegmentation is found fairly often in patients with a low serum vitamin B_{12} concentration How

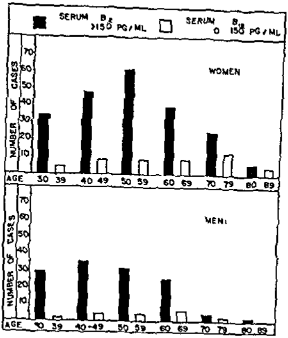


Fig 1 Age distribution and serum vitamin B_{12} concentration

method seems to be more exact for low concentrations of vitamin B_{12} such as are particularly important in the present investigation

The normal range for the serum vitamin B_{12} concentration is wide As will be seen from table III, most authors have found values from slightly above 100 pg/ml up to 1 300 pg/ml Values below 100 pg/ml are rare in a healthy population and are considered pathological if the Euglena method is used (3, 9, 13, 15, 18,) Values between 100 and 150 pg/ml are in the subnormal range and may be pathological

Therapy

In all patients where a low serum vitamin B_{12} concentration was found, vitamin B_{12} therapy was started 200 micro g cycobemin was given intramuscularly every second day for ten days and subsequently the same amount once a week for half a year Afterwards a maintenance dose of 200 micro g was given every month

Results

Vitamin B_{12} determinations

The results are given in table II Of 396 patients 23 were found to have vitamin B_{12} levels below 100 pg/ml, i e 5,8 % of the total number examined 61 or 15,4 % of the patients had values below 150 pg/ml

It seems probable that a B_{12} deficiency is found in more than 15 % of patients admitted to our mental hospital

The age distribution and serum vitamin B_{12} concentration are given in fig 1 and in table IV Fig 1 shows cases with a normal (above 150 pg/ml) or a subnormal (100—150 pg/ml) concentration of vitamin B_1 Table II shows cases with a pathological vitamin B_{12} concentration (less than 100 pg/ml) Subnormal and pathological B_{12} values are found in all groups, although the number increases with age if calculated in % of the total number in the group

Hemoglobin values

As will be seen from table V a low hemoglobin is often found in patients with low serum vitamin B_{12} level The differences between the groups are statistically significant in both sexes, but of no diagnostic value

Erythrocyte sedimentation rate

A sedimentation test has been carried out in all patients No correlation between an increased sedimentation rate and a low serum-vitamin B_{12} concentration was found

An auto immune mechanism has recently been suggested for patients with a low serum vitamin B_{12} concentra

Somatic findings	Neurological abnormalities	EEG	Effect of therapy	Remarks
None	None	Normal	+	
None	None	Generalized cerebral dysrythmia	++	Resectio ventriculi in 1952
None	None	Generalized theta activity	0	
Glossitis	None	Generalized cerebral dysrythmia	+	
None	None	Normal	0	
Glossitis	Impaired sense of vibration	—	+	Cancer ventriculi operata 1962
None	None	Normal	No therapy	Lobotomi in 1956
Glossitis	None	Low frequent disturbances	++	Achylia gast. diagnosed in 1940
None	None	Normal	0	
None	None	—	No theraphy	
None	None	Normal	0	Thrombosis cerebri Mors
Astenic	None	Normal	+−	Resectio ventriculi 1956
Glossitis	None	Normal	+−	Anemia pernic diagnosed 1940 No therapy since 1960
None	None	Normal	0	
Glossitis	None	Low frequent disturbances	−+	
Exophtalmic goiter	Impaired sense of vibration	Normal	++	Thyreotoxicosis
None	None	Paroxystic theta activity over left hemisphere	No therapy	
None	None	Low frequent disturbances	0	Fract. colli femoris Mors
None	None	Normal	−	Cancer mamma operata No metatases
None	None	Normal	0	
None	None	Normal	+++	
Astenia glossitis	Impaired sense of deep pain	Low frequent activity mainly over right hemisphere	−	
None	None	Normal	0	Hypothyreoidism for 8 years Thyroxinetreated

...ageable 0 no effect of therapy

TABLE VII A summary of the findings in patients with serum vitamin B_{12} level below 100 pg/ml

Case	Sex	Age	Serum B_{12} (pg/ml)	Psychiatric diagnosis	Duration of mental diseases
1	♂	43	74	Schizophrenia	2 years
2	♂	63	64	Manio-depressive psychosis	12 years
3	♀	42	92	Schizophrenia, catatonic. Acute delirium	5½ years
4	♀	41	49	Schizophrenia hebephrenic	4 years
5	♂	39	82	Manio-depressive psychosis	24 years
6	♀	83	75	Senile dementia	2 years
7	♂	56	96	Schizophrenia, hebephrenic	34 years
8	♀	50	93	Psychosis e constitutione, deficientia intelligentiae	6 years
9	♂	65	69	Manio depressive psychosis	42 years
10	♂	72	94	Depressive reaction	1 year
11	♀	81	58	Schizophrenia hebephrenic	24 years
12	♀	56	25	Psychoneurosis	13 years
13	♀	75	7	Schizophrenia, paranoid	15 years
14	♀	37	83	Schizophrenia hebephrenic	12 years
15	♀	64	41	Psychosis e constitutione paranoid, depressive reaction	1 year
16	♀	46	52	Psychosis e constitutione paranoid reaction	2 weeks
17	♀	81	87	Senile dementia	2—3 years
18	♀	76	34	Senile dementia	2 years
19	♀	49	68	Psychosis e constitutione depressive reaction	4 weeks
20	♀	32	38	Psychoneurosis depressive reaction	12 years
21	♀	59	76	Involutional psychosis schizophrenia?	8 years
22	♀	80	43	Schizophrenia paranoid	60 years
23	♀	60	77	Involutional psychosis paranoid reaction	7 years

Effect of theraphy + + + complete recovery + + considerable improvement + more man

Psychiatric diagnosis Duration of the mental disease

In table VII the psychiatric diagnoses for all the patients with a vitamin B_{12} deficiency are listed No specific psychiatric syndrome is found in B_{12} hypovitaminosis although paranoid symptoms are rather common

In 10 patients the psychiatric symptoms had lasted for more than 10 years — 4 of these had been continuously ill, in the others remissions had occurred On the last admission all the patients were psychotic or a relapse had occurred in the disease

Somatic and neurological findings

In 5 of the patients with a hypovitaminosis B_{12} a glossitis was found Two of the patients were in a bad nutritional condition Two patients demonstrated a distinctly impaired vibration sense in the distal part of their legs A pathological EEG was found in 8 patients In the main a generalized slow activity was found In two patients the changes were most prominent over one of the hemispheres EEG findings were in accordance with those found in pernicious anemia (30)

Results of therapy

In addition to psychiatric treatment all patients with serum vitamin B_{12} levels below 100 pg/ml received vitamin B_{12} therapy The results are given in table VII column 10 One patient recovered completely from his mental disea e 7 improved considerably, 4 patients improved to some extent and became more manageable Glossitis if found disappeared in all EEG changes persisted, however, through the observation period (several months)

Discussion

Our investigation has revealed a surprisingly high incidence of vitamin B_{12} deficiency in patients admitted to a mental hospital The incidence exceeded by far that in the general population The frequency of pernicious anemia in the area surrounding Lier Mental Hospital has been reckoned as 9,3 per 100 000 inhabitants annually (14) In 396 patients we have found 23 with a serum vitamin B_{12} concentration below 100 pg/ml None of these patients were in such a bad nutritional condition that this could account for their hypovitaminosis (7)

Only one of the patients had a pernicious anemia All the others had a hypovitaminosis B_{12} according to determinations made by the Euglena method

This microbiological method will of course respond to anything which impairs the growth of the algae Lack of vitamin B_{12} is one factor We do not know however if serum from psychotic patients contain unknown compounds which would impair the growth of the algae even in the presence of vitamin B_{12} Even normal serum may impair the growth of Euglena (3) It is known that serum from some psychotics will impair the uptake of glucose in the isolated rat diaphragm (29) The same compound could of course influence the growth of Euglena Work to elucidate this problem is in progress

As most of our patients improved on a vitamin B_{12} therapy and the serum

ever, overlapping between the groups is found frequently and the findings are thus of little practical value

Supplementary examinations in patients with serum vitamin B_{12} concentration below 100 pg/ml

Supplementary examinations were carried out in all patients with a vitamin B_{12} level below 100 pg/ml This group consisted of 22 patients — 17 females and 5 males Not all of the supplementary examinations could be performed in every patient, due to lack of cooperation One of the male patients was discharged from the hospital before a complete examination had been performed

Red blood cell count

In 10 of the 17 female patients a red cell number of less than 4,2 mill /mm³ was found and in one of the 5 male patients the number was less than 4,6 mill /mm³ There seems to be a correlation between a low red cell count and a low serum vitamin B_{12} concentration

Mean corpuscular hemoglobin (MCH)

A MCH above 31 was found in one of the 5 male patients and in 12 of the 17 female patients

Serum iron

Increased serum iron concentration (220 µg %) was found in one male patient, otherwise all values were below the upper normal limit of 170 µg % This is by no means astonishing Increased serum iron is dependent on a certain degree of bone marrow depression and is consequently only found when the hemoglobin values are lower than in our material

Investigation of the gastric juice after histamine stimulation

Achlorhydria after histamine stimulation was found in 5 male patients and in 9 of the 14 female patients In 5 patients a normal hydrochloric acid secretion was found in spite of a low serum vitamin B_{12} concentration

The Schilling test

A 24 hours excretion below 7 % was found in 11 of the 16 females tested and in 2 male patients tested The correlation between a low serum vitamin B_{12} concentration and a pathological Schilling test is good but not complete This has been found by other authors (19, 20) No contradictions are involved in this finding The Schilling test reflects only the absorption capacity for vitamin B_{12} The actual serum concentration of the vitamin depends on alimentary supply absorption, metabolic activity, the stores of vitamin B_{12} and excretion — factors which may change independently of the absorption capacity

Bone marrow examination

This investigation was carried out in 17 female and 5 male patients Only in one of the female patients there was found a typical megaloblastic bone marrow In this case the peripheral blood picture was typical for pernicious anemia In 6 cases pernicious anemia might be suspected A few megaloblasts were seen but no increase in erythropoiesis In 6 patients hypersegmentation of the granulocytes was noted

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vitamin B_{12} level increased during this therapy, it is justified to believe that most of them were in a hypovitaminotic state

In our material we have found a tendency to a decreased hemoglobin value and an increased mean corpuscular hemoglobin in B_{12} hypovitaminosis However, overlapping with a normal material is great Examination of the gastric hydrochloric acid level does not give any lead to a vitamin B_{12} hypovitaminosis Five cases with a low B_{12} level had a normal secretion Determination of serum iron and the Schilling test are also of limited value for the diagnosis of vitamin B_{12} deficiency

No specific psychiatric syndrome is found in vitamin B_{12} deficiency At the present time determination of serum vitamin B_{12} seems to be the only reliable method to reveal a B_{12} hypovitaminosis Factors which would invalidate the microbiological method must, however, be kept in mind

The connection between mental illness and vitamin B_{12} deficiency cannot be evaluated from our material It is hard to judge to what extent the improvement of mental symptoms in 8 of our patients is due to vitamin B_{12} treatment

The heterogenity of the mental symptoms in our material is by no means astonishing The symptomatology of mental diseases depends on genetic and environmental factors A metabolic lesion which affects brain metabolism would thus be expected to give a polysymptomatic picture

It is impossible from the clinical picture to screen out patients with a B_{12} hypovitaminosis

Long lasting vitamin B_{12} deficiency will produce organic damages and the result of a vitamin B_{12} treatment will be poor An early diagnosis and treatment of vitamin B_{12} hypovitaminosis is of importance

Summary

All patients above 30 years of age admitted to Lier Mental Hospital during a one-year period were investigated with regard to a vitamin B_{12} deficiency

Of the patients 256 were females and 140 males, altogether 396 In 23 (5,8 %) a serum vitamin B_{12} concentration below 100 pg/ml was found This far exceeds the frequency found in the general population

The vitamin B_{12} deficiency group did not present clinical or hematological findings which would indicate a B_{12} deficiency They could be screened out only by determination of the serum vitamin B_{12} concentration

The importance of the findings and therapy with vitamin B_{12} is discussed

Acknowledgement

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Cortisol Production Rate in Patients with Acute Bacterial Infections

By

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Different opinions appear in the literature regarding the use of *cortisone in* acute, severe infections (4 9 10 13) Cortisone therapy is however recommended during acute, bacterial infections in patients with a proven failure of the pituitary adrenocortical function

The suggested dose of cortisone is based upon the clinical impression of the course of the disease Goldthorpe and Dougherty (5) stressed the importance of an optium dose of cortisone Too large doses of cortisone may depress the resistance against infection (6a 6b 11) and too small doses may lead to Addisonian crisis

This paper is concerned with the excretion of 17 hydroxycorticosteroids (17 OHCS) and the *cortisol production* rate in patients with acute bacterial infections

Material and methods

The patients have been selected for the study of administration of 4 C¹⁴-cortisol during acute infections (table I) There was a slightly increased retention of bromsulfalein (BSP test) in patient No 4 (19 % after 45 min) No 5 developed hypotension and oliguria during the first day in hospital She was successfully treated with Metaradrini bitartras (Aramine® MSD) intravenously for four days

4 C¹⁴ cortisol with a *specific activity* of 42 4 uc/mg was obtained from the National Institutes of Health Bethesda Md , U S A 0 36 to 1 07 uc of 4 C¹⁴-cortisol was injected *intravenously Suitable aliquots of the 4 C¹⁴* cortisol solution were taken prior to injection for estimation of *radioactivity in* the injected dose Urine sampling took place 3 6 9 12 24 and 48 hours after the administration of the radioactive cortisol

The *cortisol* production rate was determined as previously described (1) In brief this entails extraction of the *urine with ether*/ethyl acetate (2 1) after glucuronidase hydrolysis *purification of the extracts through* washes with N NaOH and distilled water isolation of tetrahydrocortisone and *tetra*hydrocortisol by paper chromatography in the benzene/formamide system and the estimation of these compounds by blue tetrazolium reagent

The *radioactivity* was measured by means of a windowless gas-flow counter (*Frieseke* and *Hoepfner F H 407*) Background

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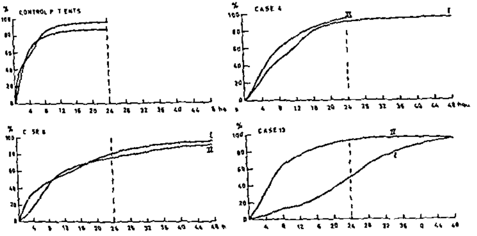


Fig 1 The cumulative excretion of 4 C[14] cortisol in two control patients and in three patients with acute bacterial infections I During the acute stage of infection II During convalescence

convalescence the values were found to be between 10 9 and 17 2 mg/24 hours The values obtained simultaneously for urinary 17 OHCS were respectively 23 7—34 0 mg/24 hours and 2 2—8 9 mg/24 hours

The relation between the cortisol production rate and the output of 17 OHCS was estimated in three patients during the acute stage of infection and in convalescence The results showed that a mean of 43 per cent of the endogenous cortisol production was recovered as urinary 17 OHCS during the acute stage of infection while 46 per cent was recovered during convalescence

Discussion

After administration of 4-C[14] cortisol the same per cent of radioactivity was found in the 24 hour urines in two patients suffering from acute severe bacterial infections as in two control persons A similar percentage recovery was also found in man by Hellman et al (7a, 7b) Peterson and Wyngaarden (16) and Plager et al (17) The pattern of the cumulative excretion of radioactive metabolites was identical during the acute stage of infection and during convalescence These results indicate that there was no major alteration in cortisol metabolism and excretion in these patients

Marks and Leaf (14) described a correlation between the renal excretion of adrenal corticosteroids and the renal plasma flow in dogs In shock due to infection there is a reduction of the urinary output of corticosteroids and a retention of conjugated cortisol metabolites in the plasma (15) A reduced excretion of cortisol metabolites was found in the present material in one patient suffering from shock due to meningococcal infection

The oldest patient in this material was 73 years old She had never suffered from liver disease but the retention of

TABLE I The endogenous cortisol production and the excretion of 17 OHCS in the patients with acute bacterial infections during the acute stage and during convalescence No 5 developed hypotension and oliguria just after the investigation had begun and was treated with Aramine® The corticosteroid estimation given is the mean from the first two days in hospital

				Acute stage		Convalescence	
No	Age (yrs)	Diagnosis	Temp on admission (C°)	Cortisol prod (mg/24 hrs)	Urinary 17 OHCS (mg/24 hrs)	Cortisol prod (mg/24 hrs)	Urinary 17 OHCS (mg/24 hrs)
1	49	Erysipelas	39 8	34 9			
2	53	Pneumonia	40 6	24 7	24 6		
3	24	Meningitis (pn)	38 7	89 8	27 7	13 7	8 9
4	73	Meningitis (pn)	39 1	60 5	34 0	17 2	8 8
5	47	Meningitis (me)	40 6	53 8	23 7	10 9	2 2
6		Control				16 5	
7		Control				17 0	

radioactivity was about 20 counts/minute 1 ml of each urine sample and suitable aliquots of ethanolic solutions of the purified steroids were dried under an infra red lamp in planchettes of area approximately 7 cm^2 The planchettes were lined with a disk of lens tissue to ensure even distribution Samples were counted for a sufficient time to ensure a statistical error of less than 3 %, and all counts were then corrected to infinite thinness by means of a self absorption curve

Total 17 OHCS in 24-hour urine samples was determined by Jorgensen s (8) modification of the method of Appleby et al (2)

Results

The excretions of radioactive cortisol metabolites in 24 hour urine samples from two control persons were 87 and 96 per cent respectively (fig 1)

In one patient suffering from meningitis (No 3) 89 3 per cent was excreted per 24 hours in the acute stage of infection When the test was repeated in convalescence, 94 2 per cent was excreted per 24 hours In another patient with meningitis (No 4) the values were 80 per cent and 78 per cent respectively The result of a BSP-test in this patient was 19 per cent retention

In a patient with meningococcal meningitis (No 5) 95 per cent of 4-C^{14}-cortisol was excreted in convalescence Delay in excretion occurred in the acute stage of infection, while the patient had transient oliguria and hypotension

The cortisol production rate during the acute stage of infection in the five patients was found to be between 24 7 and 89 9 mg/24 hours (table I) All the patients had uncomplicated recoveries In

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bromsulphalein was increased (19 % retention), as may be found in elderly patients (19). This finding may explain the slight delay in urinary excretion of 4-C^{14} metabolites.

During the acute stage of infection an increased cortisol production rate (24.7—89.9 mg/24 hours) was found. The results may indicate the doses of cortisone needed for patients with a proven failure in the pituitary-adrenocortical system in stressful conditions.

During the acute stage of infection 43 per cent of the cortisol secretion was recovered as urinary 17-OHCS. In convalescence, when the patients had cortisol rates within normal limits, 46 per cent of the cortisol was recovered as urinary 17-OHCS. There are, however, individual variations, and the accuracy of the estimations is influenced by the secretion on the day before the test (12). It appears that the percentage of secreted cortisol recovered as 17-OHCS is similar during normal and increased adrenocortical activity. Silber (18) found a linear correlation between oral doses of cortisone and the urinary excretion of cortisol metabolites.

Ten determinations of urinary 17-OHCS were carried out in four patients on replacement therapy with cortisone (3). Approximately 36 per cent of the administered dose of cortisone was excreted in 24 hour urine samples as 17-OHCS. It may be concluded as a rough guide that approximately 40 per cent of the secreted cortisol is excreted as 17-OHCS by the methods employed, both in normal and in "stressed" patients. Determination of the urinary output of 17-OHCS may give valuable information on the adrenocortical function in patients suffering from acute, severe infections. The results may, however, be invalidated by impairment of the liver or kidney function or oliguria due to infectious shock.

In these patients there was a great variation in the cortisol production rate. This findings may, however, be explained by differences in the duration of the disease. Patients with a history of infection lasting more than one week may have normal adrenocortical function regardless of the height of body temperature (3).

Summary

C^{14}-cortisol was administered to five patients suffering from acute infections, and repeated in three of these during convalescence. The cumulative excretion curve of 4-C^{14}-cortisol metabolites was investigated in three patients. The curves in two patients were identical in the acute stage and in convalescence. A delay in excretion occurred in one patient with Waterhouse-Friderichsen's syndrome with hypotension and oliguria. It was found that approximately 40 per cent of the secreted cortisol is recovered in the urine as 17-OHCS. The findings were similar in adrenocortical hyperfunction and in normal conditions. It is concluded that the output of 17-OHCS may give valuable information on the adrenocortical function in patients suffering from acute severe infections.

In the present study the maximum cortisol production rate in patients with severe infection was found to be 90 mg/24 hours.

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Studies on Viral Encephalitis

By

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Viral infection may give rise to different clinical syndromes of the central nervous system (CNS) the most important of these being aseptic meningitis, encephalitis and the syndrome of paralytic poliomyelitis Wallgren's aseptic meningitis is well known (30) Although it is generally of viral etiology the original definition does not exclude certain other etiological possibilities Patients who present more deep seated neurological involvement are classified as having encephalitis or paralytic poliomyelitis Because of the borderline cases classification among the different syndromes may sometimes be difficult

Viral meningitis and viral encephalitis are often used today as the names of clinical syndromes when every other etiology has been excluded as far as possible The designations are based on clinical observation and when available on pathological studies (2) The term viral encephalitis in the heading of this paper thus refers to encephalitis of viral or presumed viral etiology It should be remembered that even where the best virological facilities are available it is only in 1/2—2/3 of the cases classified as viral meningitis and viral encephalitis that the specific etiology is established (17, 18 21) The same virus may cause different syndromes of the CNS However certain agents are more prone to produce severe disease than others It follows from this that encephalitis and aseptic meningitis often differ in epidemiology (17, 18 21) There may even be considerable differences between countries

This study concerns only viral encephalitis in which it is impossible to establish a specific etiological diagnosis on the basis of clinical signs and symptoms and in which the other symptoms are overshadowed by the neurological ones It does not consider postinfectious encephalitis which develops during the course of or after well known viral diseases with characteristic clinical symptoms The epidemiology etiology and clinical features of viral encephalitis are

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14 of the patients were from Helsinki Many of the Helsinki cases may, however, have been taken ill or caught the infection outside the capital Two members of the family in 2 families contracted encephalitis in addition to which there were several instances of aseptic meningitis in the family besides the case of encephalitis The number of cases of aseptic meningitis treated in the hospital has been 10 times that of encephalitis in the last 10 years

Fig 1 shows the age and sex distribution of the patients Fifty five per cent were males and 53 per cent were over 15 years of age The month of the onset of the disease is shown in fig 2 according to which the cases of encephalitis were distributed fairly evenly throughout the year

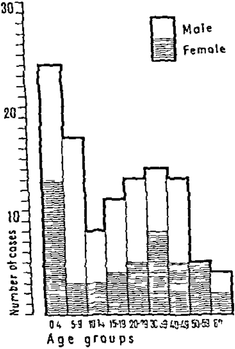


Fig 1 Age and sex of 108 patients

Clinical features

The fever lasted for 1 to 7 days in 53 cases for 8 to 14 days in 31 and over 2 weeks in 24 cases i e 22 per cent of the total and lasted for over one month in 11 of them A complication may have been the reason for the long duration of fever in at least 6 patients The fever was diphasic in one fourth of the patients

Headache was complained of by most patients capable of reporting on their condition The case reports of 5 patients carry an entry of general aching of the muscles and limbs Two-thirds of the patients had vomiting or nausea

Upper respiratory tract symptoms were present in one third of the patients within 10 days before the onset of the neurological symptoms The onset coincided with the encephalitic symptoms in 5 of these patients and preceded them by 1 to 10 days in the other 31 A further 10 patients had had such symptoms 11 to 21 days before the onset of encephalitis Most cases involved cough or corrhyza, but only a sore throat was involved in one third of the patients Mild fever sometimes occurred The respiratory symptoms had often disappeared or were about to disappear when the encephalitis began Pneumonia was established in 6 patients in the initial phase of encephalitis The X-ray findings in 3 cases were of the primary atypical pneumonia type the other cases had bronchopneumonia Five patients had diarrhea which began in 3 of them concurrently with the neurological symptoms Two patients had it only during the first phase of the diphasic

studied on the basis of cases seen at the Aurora Hospital Aseptic meningitis treated in this hospital has been the subject of many papers (9, 10, 14, 25) A study dealing with the epidemiology of viral meningitis and encephalitis in the whole country serves as a background to the present study (16)

Material and methods

Criteria for inclusion in the study

The material consists of 108 patients with viral encephalitis treated at Aurora Hospital in 1945—1963 This hospital admits the majority of patients taken ill with meningitis and encephalitis in Helsinki which has a population of about half a million Most of the patients have been treated by the authors personally Only the cases with acute febrile onset are included

The cases were classified as encephalitis on the basis of the same criteria as were employed by Meyer et al (21) Most of the patients had serous meningitis in addition to encephalitic symptoms Severe disturbances of consciousness e g coma convulsions pathological reflexes, mental deterioration personality changes tremor or ataxia often in combination presented clinical evidence of a more deep-seated involvement of the CNS However, convulsions of short duration occurring during the fever were not considered to warrant a diagnosis of encephalitis without other signs mentioned above when the patient was under 2 years of age or without meningitis when the patient was over 2 Patients with manifest encephalitis who also demonstrated flaccid or spastic paralysis were classified as encephalitis Patients who had the syndrome of paralytic poliomyelitis according to the criteria published by the World Health Organization (6) were not included in the material

Virological studies

The material comprises 77 cases from 1952 onwards at which time virological studies were gradually begun The number of patients admitted from 1958 onwards totals 34 These patients were subjected to a large number of serological tests, and routine examination of stool samples Before 1958, stool samples were examined in only 16 cases

Virus isolations

During 1952—58 virus isolations were performed with the aid of tissue cultures of primary human embryonic fibroblasts and from 1957 in some cases also in primary human amnion cells From 1958 all stool samples were routinely inoculated into primary human amnion cells, U cells (a continuous amnion cell line) and from 1960 onwards into primary monkey kidney cells and into newborn mice, as described earlier (14)

Serological tests

Up to 1957, complement fixing antibodies (CF) were determined only against parotitis and poliovirus types 1—3, but since that year also for influenza A_2 and B, adeno herpes simplex, Eaton agent parainfluenza 1, reovirus, ornithosis choriomeningitis lymphocytica (LCM), Coxsackie B3 and 5, encephalomyocarditis and respiratory syncytial virus Some of the latter determinations have later been made on serum samples stored at −20°C Since 1957, hemagglutination inhibition tests for tickborne encephalitis (TBE) have been carried out Insofar as serum quantities allowed neutralization tests were also performed in primary human fibroblasts for ECHO 5 6 and 9

Serological tests were performed in all cases for syphilis and after 1952 in most cases for leptospires Dye and CF tests for toxoplasma were carried out in most cases since 1958 and before that year only occasionally

Results

Epidemiology

There have been 2 to 10 cases an average of 5 to 6 per year Despite annual variations the incidence seems to have remained more or less constant All but

14 of the patients were from Helsinki Many of the Helsinki cases may, however, have been taken ill or caught the infection outside the capital Two members of the family in 2 families contracted encephalitis, in addition to which there were several instances of aseptic meningitis in the family besides the case of encephalitis The number of cases of aseptic meningitis treated in the hospital has been 10 times that of encephalitis in the last 10 years

Fig 1 shows the age and sex distribution of the patients Fifty five per cent were males and 53 per cent were over 15 years of age The month of the onset of the disease is shown in fig 2 according to which the cases of encephalitis were distributed fairly evenly throughout the year

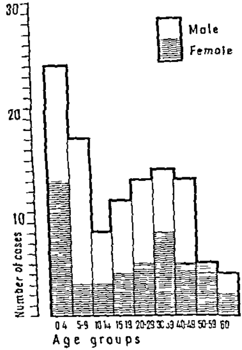


Fig 1 Age and sex of 108 patients

Clinical features

The fever lasted for 1 to 7 days in 53 cases for 8 to 14 days in 31 and over 2 weeks in 24 cases i e 22 per cent of the total and lasted for over one month in 11 of them A complication may have been the reason for the long duration of fever in at least 6 patients The fever was diphasic in one fourth of the patients

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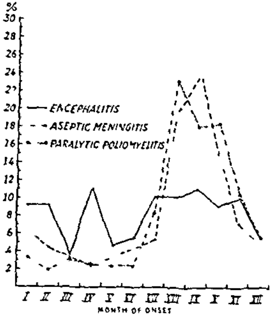


Fig 2 Seasonal distribution of cases of encephalitis as compared with cases of aseptic meningitis and paralytic poliomyelitis treated at the same hospital (10)

Table I Incidence of some neurological signs

Sign	< 15 yrs (%)	> 15 yrs (%)
Disturbances of consciousness	52	41
Mental disturbances	32	17
Tonus disturbances	42	25
Kinetic disturbances	42	20
Paralyses	38	34
Convulsions	30	4

disease Three cases had stomatitis, there was one fatal case in which severe stomatitis began before the encephalitis Nine patients had an uncharacteristic rash in the initial phase of the disease, 5 had conjunctivitis and 4 had transient stasis of optic discs

In one third of the patients the highest ESR was less than 15 mm, in one-third it was 15 to 30 mm and in the rest more than 30 mm The highest leukocyte count was less than 10,000 in 59 cases 10,000 to 15,000 in 20 cases and more than 15,000 in 26 cases

The highest spinal fluid cell count per mm³ was as follows (no of patients in brackets) 0—5 (22), 6—10 (4), 11—100 (35), 101—300 (29), 301—1033 (18)

Pleocytosis was thus absent in 20 per cent of the patients It was not uncommon for polynuclear cells to dominate in the first examination Lumbar pressure was usually normal or slightly elevated, being considerably elevated in a few cases only Glucose content of the CSF was normal Spinal fluid protein content was normal or moderately elevated Values in excess of 200 mg/100 ml were encountered in only 3 patients

Table I shows the incidence of some *neurological signs* in the different age groups About one third of the patients had paralysis, mainly bulbar or bulbospinal, in addition to encephalitic manifestations Seventeen patients (15 %) complained of vertigo, nystagmus was established in 7 of them Meningitic symptoms dominated the clinical picture of 16 patients most of them adults The EEG changes will be discussed in a study on sequelae On discharge from hospital one-third of the patients had neurological manifestations

Fatal cases

Eleven patients i e 10 % died Five of them were under 15 In only 2 patients had respiratory symptoms preceded the neurological condition The fever was diphasic in 3 cases The course of the

TABLE II Results of virus isolations and antibody assays in patients with viral encephalitis

Etiological agent tested for	No of pat	Probable etiology established[1]	
		No	%
Mumps	50	4	8
Tick borne meningo-encephalitis	42	5	12
Herpes simplex	34	2	6
Poliomyelitis	50	2	4
ECHO	50	2	4
Coxsackie B	34	1	3
Adenovirus	30	3	9
Influenza	30	1	3
Ornithosis	30	1	3

[1] Isolation of virus and/or significant rise of antibody titer

disease was fulminant in the majority of the cases Seven patients died 2—5 days after the onset of the disease Four patients did not die until 2—4 weeks after the onset of the disease Tracheotomy was performed and an intermittent positive pressure respirator was used in 3 fatal cases This type of treatment often seems to prolong the moribund state with the result that autolysis of the brain makes evaluation of the microscopical findings in the cases difficult However there were 2 cases in our series in which the respirator treatment may have been lifesaving Autopsy was performed in all the cases which ended fatally but the results are difficult to assess in the absence of systematic serial sections Microscopical study revealed capillary congestion and in most cases perivascular collar like lymphocyte and plasma cell infiltration especially in the region of the basal nuclei Prominent focal demyelinative lesions were not demonstrated in a single case

Virology

Cytopathogenic agents were isolated in 1954 and 1955 in two cases These agents were not neutralized by antisera against polioviruses and did not infect newborn mice Since there was no possibility of identifying these viruses, they were considered to be ECHO viruses Type 3 polioviruses were isolated from stools in 2 nonparalytic cases in 1956 and 1957 From 1958 onwards stool samples from every patient and spinal fluid samples from many cases were examined in at least three kinds of tissue cultures and in newborn mice, but only two strains were isolated Coxsackie B5 was discovered in one case and adenovirus type 7 in a case with pneumonia

Infection was also considered or suspected to be associated with a specific agent if a fourfold or greater increase of antibodies was found in CF hemagglutination inhibition or neutralization tests In mumps a CF antibody titer of 1 64 or higher without any increase was inter

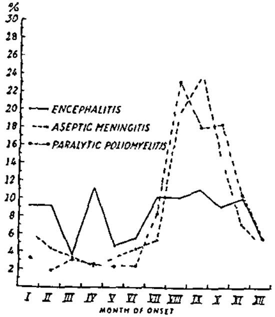


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indicate the etiology the diagnosis was arrived at in 40 % of the cases with encephalitis examined from 1958 on wards while the corresponding figure has been over 30 % for aseptic meningitis

Discussion

Viral encephalitis is a relatively un common syndrome However, in prac tice this diagnosis is often used for cases which are not viral diseases of the CNS or which should not be classified as encephalitis (16) On the basis of the present work and an earlier epidemiologi cal study covering the country as a whole (16) the annual incidence per 100,000 inhabitants can be estimated at 2—3 cases As the number of cases of aseptic meningitis treated in hospital in the last 10 years has been 10 times that of encephalitis, the real incidence of meningitis must obviously have been relatively higher since many cases may be treated at home often without being correctly diagnosed Epidemiologically interesting is the observation that en cephalitis is of roughly equal frequency in the different seasons while the peaks of aseptic meningitis and paralytic polio myelitis occur in late summer and autumn The different etiological group ing of meningitis and encephalitis must be the reason for the difference Entero viruses which are known to be diseases primarily occurring in the warm season of the year play a very much smaller role in the etiology of encephalitis than in that of aseptic meningitis In contrast it appears that respiratory viruses which cause diseases above all in the cold part of the year, may be of relatively greater importance in the etiology of encephalitis than of aseptic meningitis

A probable etiological diagnosis was arrived at in 40 % of the patients with encephalitis from 1958 onwards The etiology of viral encephalitis was estab lished by Lennette and his co workers in 45 % (17, 18) and by Meyer in 64 % of his cases (21) As the etiological diagnosis of aseptic meningitis was established considerably more frequently than that of encephalitis, the agents causing encephalitis include a greater number of viruses that we are at present incapable of identifying Eleven per cent of the present cases of encephalitis were of enteroviral etiology while the com parable figure for aseptic meningitis has been 43 % in the last few years and earlier during the time of poliomyelitis, even higher Our results agree fully with those of Meyer who reported 7 % of 139 cases of encephalitis and 39 % of 430 cases of aseptic meningitis to have been caused by enterovirus (21)

Among the viruses causing diseases of the CNS in the warm period of the year the tick borne encephalitis virus must be borne in mind *in addition to the* enteroviruses This illness has been the subject of comprehensive studies in Finland in the last few years (15 23 24 29) It has been noted that the infection manifests itself more frequently as aseptic meningitis than as encephalitis Mumps virus seems to play an equally great role — about 10 % — in the etiology of both encephalitis and aseptic meningitis which agrees with observations made elsewhere

TABLE III. Results of virus isolations and antibody assays in patients with aseptic meningitis

Etiological agent tested for	134 patients 1952—55 No. of pat.	134 patients 1952—55 Probable etiology established[1] (%)	113 patients 1962—63 No. of pat.	113 patients 1962—63 Probable etiology established[1] (%)
Poliomyelitis	85	42	109	—
ECHO	85	18	109	21
Coxsackie	27	4	109	22
Mumps	79	14	109	9
Herpes simplex	35	3	109	—
Tick borne meningo-encephalitis	—	—	98	2
Adenovirus	—	—	102	2
(Leptospira)	106	5	104	—

[1] Isolation of virus and/or significant rise of antibody titer.

preted as indicative of recent infection. On these criteria the serological tests pointed to a specific etiology in the following 19 cases (table II):

1. Mumps in 4 cases.
2. TBE in 5 cases. Three patients had contracted the disease after tick-bite in endemic areas and two were laboratory infections. All were relatively mild diseases without paralysis.
3. Herpes simplex in 2 cases, one of which was fatal. Concurrently with the encephalitis this patient had severe stomatitis. Autolysis of the brain — as mentioned earlier — made microscopical evaluation difficult and may have contributed to the failure to isolate the virus.
4. Poliovirus type 3 in the 2 cases mentioned above.
5. Coxsackie B5 in the one case mentioned above.
6. Adenovirus infection in 3 patients, all of whom had upper respiratory tract symptoms. One of these was the pneumonia patient, from whose stools adenovirus type 7 was isolated. In this and one of the other cases the titer rose to 1:256.
7. Influenza A in one case with bronchopneumonia. At the same time — in April 1960 — there were several virologically diagnosed influenza A_2 infections in Helsinki without a widespread epidemic.
8. Ornithosis in one fatal case.

In one of the cases of encephalitis examined for leptospirosis (corresponding in 2 %), a high antibody level was found. This case is not included in our material.

Table II presents the results of virus isolations and antibody assays of the patients tested. The corresponding data of the patients with aseptic meningitis treated at the hospital in 1952-55 (25) and 1962—63 are shown in table III.

In as much as it can be considered that virus isolations and/or increase of the antibodies during the disease reliably

to isolate a respiratory virus from the spinal fluid or from the brain (3, 7) Since it is often impossible to type a case of encephalitis as virogenic or as a neuroallergic reaction (26, 28), we have felt it best from the clinical point of view to include in our series all those cases of encephalitis — whatever their pathogenesis — in which a specific etiological diagnosis was impossible on the basis of the clinical symptoms This step is also warranted theoretically since there is now a good deal of evidence to suggest that a hypersensitive or allergic reactivity of the tissues to the virus is important even in the pathogenesis of viral encephalitis characterized by viral invasion of the CNS and absence of demyelinating lesions The latest link in this chain of evidence is the interesting observation that neonatal thymectomy protects mice from the lethal effects of an intracerebral inoculation with choriomeningitis lymphocytica virus (19)

Summary

The epidemiology etiology and clinical picture of viral encephalitis on the basis of 100 patients treated in Aurora Hospital in 1945—1963 was studied A specific etiological diagnosis was impossible on the basis of the clinical symptoms Aseptic meningitis treated in the same hospital was used for comparison

The annual incidence of viral encephalitis appears to be 2—3 cases per 100 000 inhabitants that of aseptic meningitis over 10 times higher The incidence of encephalitis is roughly the same in the different seasons of the year, but aseptic meningitis and paralytic poliomyelitis are most frequent in late summer and autumn From 1958, a definite or probable etiological diagnosis was arrived at in 40 per cent of patients with encephalitis, while the corresponding figure was over 50 per cent for aseptic meningitis before and after this year Enteroviruses play a much smaller etiological role in encephalitis than in aseptic meningitis Mumps, TBE, herpes simplex and some respiratory viruses, above all adenoviruses, are among the etiological agents identified The results of our virological studies and the observation that one third of the patients had upper respiratory tract symptoms within 10 days before the onset of encephalitis argue in favour of the assumption that respiratory viruses play a prominent part in the etiology of encephalitis in Finland This together with observations on the etiological significance of enteroviruses accounts for the difference in seasonal distribution of encephalitis and aseptic meningitis Neurological symptoms and signs in different age groups are discussed In one third of the patients there were still patho-neurological manifestations on discharge from hospital The mortality rate was 10 per cent Autopsy was performed in all the fatal cases Demyelinating lesions were not demonstrated in a single case

Acknowledgement

This investigation was supported by grants from the Sigrid Jusélius Foundation and the Rockefeller Foundation

One-third of the patients with encephalitis had cough, corrhyza or sore throat within 10 days before the onset of the neurological symptoms The comparable figure for aseptic meningitis treated in hospital was 20 % (10) Presumably the neurological and respiratory symptoms are usually caused by the same respiratory virus It is possible, of course, that a coincidence of two infections of diverse etiology is sometimes involved This does not, however, rule out the possibility that both infections might contribute to the genesis of the CNS disease The respiratory symptoms may also occasionally be caused by a "non respiratory" virus The results of our virological studies, together with the commonness of respiratory symptoms, argue in favour of the possibility that respiratory viruses, especially the adenoviruses, may play an important role in the etiology of encephalitis As the diagnosis in these cases — with the exception of one case which was published previously (11) — is based only on serological tests and the presence of respiratory symptoms, no final conclusions can be drawn It is well known that during some viral infections anti bodies may develop not only against the causative agent but also against other, often antigenically related viruses (4, 8, 20) Case reports in which the CNS disease may have been caused by an adenovirus have been published recently in various countries (3, 5, 11 13, 18, 22, 27) Viruses as yet unknown or about which we know little such as rhinoviruses, may be important The etiological agent should be sought, at least in cases with respiratory symptoms, not only from the stools but also from throat swabs The presence of virus in the spinal fluid and in fatal cases in the brain should also be studied

Clinically, only cases with an acute febrile onset are included in our series We know, however, that there are also cases with apyrexial viral encephalitis, but their differential diagnosis, e g from multiple sclerosis, is very difficult Our study gives some idea of the multiplicity of neurological symptoms and the different clinical manifestations of the disease in children and adults One-third of our patients suffered from paralysis, compared with 27 % in Meyer's series (21) Fifteen per cent complained of vertigo and at least half of these had nystagmus The pathogenesis of these symptoms may be diverse The mortality rate was 10 %, i e the same as in Meyer's study It was not possible in a single case to establish demyelinating lesions

Our material contains a considerable number of cases of encephalitis which may be called postinfectious to a non specific respiratory illness or to an alimentary upset In many cases, however, the encephalitic symptoms arose a very short time after the onset of the respiratory or alimentary symptoms and sometimes simultaneously with them It is well to remember that prodromal respiratory symptoms are also a frequent feature of the diseases of the CNS caused by the non respiratory viruses that invade the CNS and cause a primary neurone injury (1, 12) Although the demyelinating lesions that are characteristic of so called postinfectious encephalitis have often been found in fatal encephalitis following a respiratory illness, it has in some cases of encephalitis been possible

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From the Renal Ward Fourth Medical Department (Head B von Bonsdorff, M D) University of Helsingfors Finland

The Therapeutic Success and Nephrotoxicity of Colistin in Acute and Chronic Nephropathies with Impaired Renal Function

By

Leif G Tallgren, Kristian Liewendahl and Börje Kuhlbäck

Colistin is a basic polypeptide antibiotic isolated by Koyama et al in Japan (13) Colistin has recently been shown to be identical with polymyxin E (29) All the polymyxins i e A B C D and E have a similar antibacterial spectrum Only quantitative differences have been noted *in vitro* (3) The antibacterial actions of colistin and polymyxin B are practically identical (11) With the one noteworthy exception of *Proteus* all Gram negative bacilli are gravimetrically more sensitive to the polymyxins than to any other antibiotic (5 8 29) Pathogenic Gram negative cocci all species of Gram positive bacteria and fungi are very resistant

The preparations commercially available are polymyxin B generally simply called polymyxin and polymyxin E named colistin (colomycin) Both of them are available as the sulphate or the sulphomethyl derivative known as the methane sulphonate One mg of pure colistin base is equivalent to 30 000 Japanese units One mg of pure polymyxin B base represents however 10 000 international units

The polymyxins are excreted mainly by the kidneys There is an astonishing delay however, before the urine concentration reaches a maximum In one study only 0 1 per cent of a parenteral dose of 3 mg polymyxin B or E per kg body weight was recovered in the urine in the first 12 hours There was then a progressive increase from 40 to 400 µg/ml for the next 24 hours (24) Methane sulphonates are excreted more rapidly None of the polymyxins are absorbed from the alimentary tract

All the polymyxins are potentially nephrotoxic to the epithelium of the convoluted tubules The considerable nephrotoxicity of polymyxin A C and D precludes their clinical use Tissue damage at the site of injection is a side effect of the sulphates but is eliminated

Submitted for publication December 17 1964

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cultures was our cr ter on of successful anti bacter al therapy

All the laboratory exam nat ons were per formed n the Laboratory Department of the hosp tal (Head Docent Ralph Grasbeck M D)

Methods

Col s n methane sulphonate (Col mycin®) was adm nistered as an intramuscular nject on Attempts were made to set the dose according to the degree of renal impa rment and type of renal d sease (table I) (16) In chron c nephropath es plasma creat n ne was the sole cr ter on In add t on the cl n cal p cture as well as the ur nary output were cons dered n pat ents with acute renal disease Other potent ally nephrotox c drugs were removed before n t at on of the col st n t eatment Only n trofuranto n dosage 25—150 mg per day depend ng on renal funct on (4 27) was adm nistered s mul aneously twelve t mes and Sulfpral® (comb nat on of sulphapyridaz ne and sulphameth zole AB As ra Sweden) 2—4 tablets a day tw ce 31) The average durat on of col st n treatment was 8 5 days If a threa en ng rise in the plasma crea n ne levels occurred col st n trea ment was nterrupted w th n three days regardless of therapeu c success

Case reports

Ca e 1 A 66-year-old female ho after re c rring bacteriurias over a per od of n ne years had ma n a ned a stable renal funct on w h plasma cre n ne 2 mg 100 ml for two years Ow ng o pers s en om ng and nausea a s ate of decompensat on rap dly arose She was adm ed n uraem c pre oma w h s gns of 1 hydra on Blood pressure 200 110 mm Hg plasma crea n ne 3 35 mg 100 ml and col u a Af er proper hyd a on and arrest of om ng her plasma creat n ne returned to 2 4 mg 100 ml A empted an bac er al therapy fa led Col s n trea ment w 1 6 000 000 I U da ly was ins u ed The plasma creat nine began to rise and af er two determ nat ons yielded values of 4 50 and 4 64 mg/100 ml the treatment was interrupted The col nfect on had been replaced by *P oteus* wh ch was el m nated by chloramphen col and tetra cycl ne Twenty-eight days later the coliuria reappeared W th sod um b carbonate and kanamyc n 0 5 g every oth r day for a week her urine became abacter al but the creati n ne level has not yet returned to the in t al basel ne (fig 1)

Case 2 A 60-year-old female who since her teens had suffered from frequent attacks of m gra ne had been a regular taker of phenacet n-conta n ng analges cs About 10 years ago pyelonephrit s and an elevated blood pressure were d agnosed *F ve years* ago her plasma creat *n ne* was 3 9 *mg/100* ml Recently the bacter uria recurred Long term treatment fa led After admiss on her hypertens on was treated w th *methyldopa* 250 mg three t mes da ly and a salt free d et The pressure fell from 240 160 to 175/120 mm Hg A polymyx n B-sensit e *E col* was cul ured *from* h *ur ne* She was g ven 3 000 000 I U col st n as well as 50 mg n trofuran o n da ly She became abacterial n a couple of days but the *plasma* creat n ne rose from 4 35 to 6 8 mg/100 ml Furthermore the blood pressure s multaneously rose to 225/140 *mm Hg* but rap dly returned to 160 *110 when* chloroth az d in doses of 2,0 mg

TABLE I A erage colist n dosage in 1×10⁶ I U per day adm nistered in this ser es in relat on to actual plasma-creat n ne level

Plasma creat n ne (mg/100 ml)	<3	3—5	>5
Chron c pyelonephrit s Chron c glomeruloneph r us	4 4	3 8	2 0
Acute tubular necros s *Acute glomerulonephritis*	4 0	3 7	3 8

by the use of methane sulphonates Insignificant neurotoxic side-effects, such as paraesthesias, dizziness and weakness, may occur (1, 8)

This *report discusses* the nephrotoxic side-effects and antibacterial success of colistin methane *sulphonate in* the treatment of resistant urinary tract infections occurring in azotaemic patients Only cases in which sufficient attention was given to nephrological aspects are included

Material

A total of 25 patients, *12 men and 13 women*, were treated during the period 1962—64 Their ages ranged from 14 to 66 years The patients were distributed among the different renal disease groups as shown below

	Patients
Chronic non obstructive pyelonephritis	12
Acute tubular necrosis	10
Chronic glomerulonephritis	2
Acute glomerulonephritis	1

In all the disease groups except for chronic non obstructive pyelonephritis, the bacteriuria was *secondary to the major renal* disease All acute renal failures were due to some major extra renal disease or trauma Renal function was impaired in all patients One patient with actue tubular necrosis and one with chronic glomerulonephritis expired from their primary diseases The patient with acute glomerulonephritis died because of a secondary *staphylococcal infection* The average observation time was 32 days The nephrological diagnosis was verified histologically by renal biopsy and in cases of fatal outcome at necropsy The maximum plasma creatinine level at the beginning of colistin treatment was 13 mg/100 ml in acute cases and 8 4 mg/100 ml in chronic cases

Laboratory methods

The nephrotoxicity of colistin was followed mainly by studying the variations in plasma creatinine level Proteinuria, urinary sediment and renal histopathology were observed but *the variations were not consistent* Significant quantitative bacteriuria and an increased number of leucocytes in the urine were criteria for the diagnosis of urinary tract infection

The plasma creatinine was determined by the method of Folin and Wu (9) as modified by Brod and Sirota (2) The normal values for adults in our laboratory are 0 7—1 4 mg/100 ml The standard error of a single de*termination in the normal range is 0 017 mg*/100 ml (about 2 %) (14) In acute nephropathies determinations were performed daily, while in chronic cases they were done approximately three times a week

Qualitative bacteriological examinations were made from centrifuged urine with the aid of Gram staining and routine culture techniques Antibacterial sensitivity tests were performed according to the disc method described by Ericsson (7) He also manufactured the discs, which are supplied in Finland through AB Distra Helsingfors The cultures were made on solid media at three different pH values 5 5, 7 0 and 8 0 The standard error of one disc determination on solid media and at the mentioned pH levels in our laboratory is $\delta = 0.82$ mm (inhibition *diameter for polymyxin* B, 62 *d f*) (26) Quantitative cultures were made from clean mid stream urine in both males and females according to Marple (22) and Kass (12) More than 100 000 bacteria per ml urine was taken as significant bacteriuria Less than 10 000 bacteria per ml urine in an untreated patient was regarded as contamination In cases with *an in-dwelling catheter* a sterile closed drainage system was used (20) In these cases urine samples were taken by sterile puncture of the drainage tube Urinary sediment was semi-quantitatively examined by high power field microscopy More than four leucocytes per five fields was considered a pathological finding (18) *Routine examination of* the urine was done every other day, covering at least semi quantitative estimation of the sediment and Gram staining Sterile urine in repeated

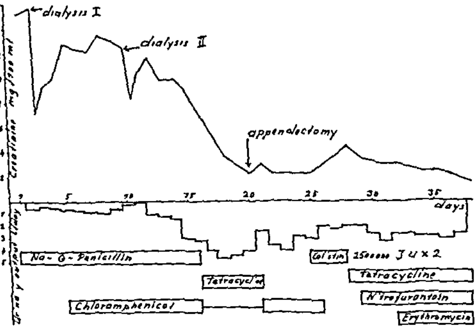


Fig 3 Case 3 Acute glomerulonephritis

daily was added The plasma creatinine however continued to rise and attained 8 2 mg/100 ml but started to fall when the blood pressure was permitted to rise Later *Proteus* appeared in her urine but was rapidly eliminated with large doses of Na benzylpenicillin The patient was then transferred to another hospital In a follow up about one year later her plasma creatinine remained steadily at about 6 mg/100 ml (fig 2)

Case 3 A 19-year-old psychopathic boy had staphylococcal pneumonia He received appropriate treatment at another hospital but disappeared from the ward in the course of treatment Two months later he got a backache and became dysuric He was admitted to the renal ward with anuria and symptoms of acute uraemia The plasma creatinine was 15 7 mg/100 ml and the blood pressure 160 120 mm Hg *Staphylococcus aureus* was found abundantly in his urine which became sterile with Na benzylpenicillin 5 000 000 I U daily Haemodialysis was performed twice after which he became polyuric The pressure fell to normal and the plasma creatinine started to fall Aspiration pneumonia followed a convulsive seizure It was rapidly cured A week later there were abdominal signs of acute appendicitis However an unaffected appendix was found at appendectomy Thereafter the patient developed a urinary infection with a nosocomial *Klebsiella* strain sensitive only to polymyxin B and kanamycin He was given 5 000 000 I U colistin daily The plasma creatinine rose in three days from 2 65 to 4 60 mg/100 ml The treatment was interrupted The clinical course started to deteriorate Later the temperature began to rise The first renal biopsy showed subacute glomerulonephritis. Cocci were seen in the second renal biopsy specimen Staphylococci were soon cultured from the blood and the patient died of staphylococcal endocarditis Metastatic staphylococcal foci were found in the lungs and kidneys at necropsy (fig 3)

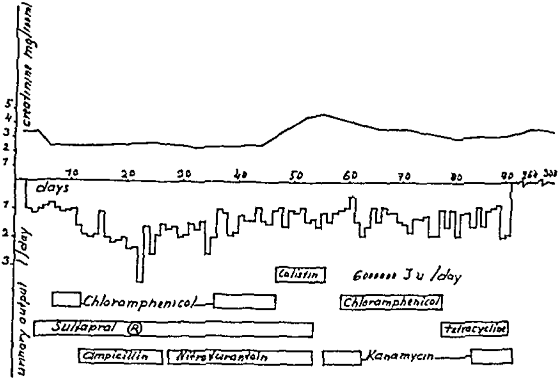


Fig 1 Case 1 Chronic pyelonephritis

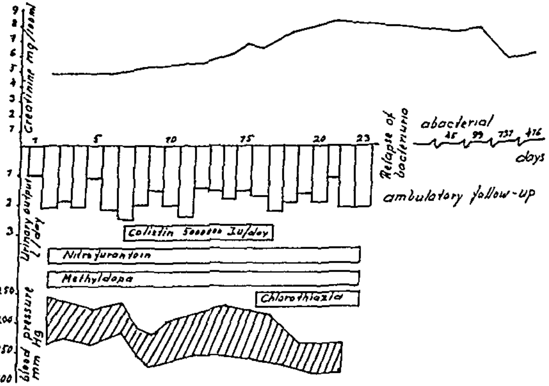


Fig 2 Case 2 Chronic pyelonephritis

TABLE IV Antibacterial sensitivity of treated organisms in vitro

No of effective antibiotics[1]	1	2	3	4	Polymyxin B according to degree of sensitivity		
					+++	++	+
Tested organisms and their number							
E. coli	10	5	5	1	17	3	1
Klebsiella	2	1	2	1	3	2	1
Pseudomonas	0	4	0	0	3	1	0
%	39	32	23	6	75	19	6

[1] Chloramphenicol chlortetracycline, oxitetracycline streptomycin kanamycin ampicillin.

TABLE V Therapeutic success and changes in bacterial flora during colistin therapy

Pathogen treated	Success of colistin therapy		Failure	Complication	
	No	Average time (days) to obtain sterile urine	No	Secondary pathogen	No
E. coli	18	4 2	3	Proteus Enterococcus	7 2
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[1] Treatment interrupted after three days in one case

to six major antibiotics About 94 per cent of the bacterias were resistant to at least three of them They may be pre sumed to be nosocomial strains

The success of colistin treatment is evident from table V as well as the secondary bacteriuria after treatment Some details not included in this table must be added In one case of chronic pyelonephritis there was a relapse with the same *Klebsiella strain* within four days In two further cases a return of the same coliuria appeared after 1 and 1 1/2 months respectively In a case of chronic glomerulonephritis with mixed bacteri uria (*E coli and Pseudomonas*), coliuria persisted until hospitalization ended in death The development of secondary infection due to colistin resistant organ isms occurred in 10 cases (40 %) 7

TABLE II Plasma-creatinine levels during colistin therapy

Changes in plasma -creatinine levels	Chronic cases			Acute cases		
	Chronic pyelonephritis	Chronic glomerulonephritis	Average colistin dosage 1×10⁶ I U per day	Acute tubular necrosis	Acute glomerulonephritis	Average colistin dosage 1×10⁶ I U per day
Increase						
Permanent	2		5 5			
Transient	3		5 0	3	1	4 1
Unchanged	4	2	3 2			
Retarded decrease				4		4 2
Decrease	3		3 9	3		3 2
	12	2	4 1	10	1	3 8

Results

Table II summarizes the changes in the plasma-creatinine level during colistin treatment Only variations larger than the calculated standard error of the method are considered The relation be tween these variations and the average dose was studied The variations have been grouped as permanent or transient increase, unchanged, retarded decrease or decrease We have tried to exclude alterations secondary to progressive anaemia, dehydration, rapid pressure fall or hypotension, post renal oliguria congestive heart failure or overdosage of digitalis and infection A rapid rise in the plasma-creatinine level in spite of suppressed or eradicated bacteriuria suggests a mechanism other than progression of the infection or parenchymal scarring

The bacteriological findings are presented in tables III—V Table III shows a random sample of 307 bacterial organisms tested *in vitro* in our laboratory The antibacterial activity of polymyxin B was not influenced by pH variations In table IV the antibacterial sensitivity of the bacterial organisms treated in this series is seen The severity of the bacteriuria is characterized by its resistance

TABLE III Susceptibility to polymyxin B in vitro of 307 different bacterial strains

	Absolute No of each species	%
Pseudomonas	29	96
E coli	129	94
Klebsiella	28	92
B proteus	62	54
S aureus	27	26
Enterococcus	38	4

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Changes in plasma creatinine levels	Chronic cases: Chronic pyelonephritis	Chronic glomerulonephritis	Average colistin dosage 1 × 10⁶ I U per day	Acute cases: Acute tubular necrosis	Acute glomerulonephritis	Average colistin dosage 1 × 10⁶ I U per day
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Permanent	2		5 5			
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cases with chronic pyelonephritis and 3 with acute tubular necrosis In all cases neither *Proteus* nor *Enterococcus* had been cultured from the urine before treatment

The variations in proteinuria were small In 2 cases with chronic pyelonephritis and one case with chronic glomerulonephritis a *slightly* increased proteinuria was noted A case of nephrotic glomerulonephritis showed a marked increase from 7 5 ‰ to 26 ‰ with return to 11 5 ‰ after withdrawal of colistin In acute nephropathies increased proteinuria was not encountered

Discussion

Bacteriology

The high antibacterial efficacy of colistin treatment was partly due to the selection of infections the causative organism of which had proved highly sensitive *in vitro* (table IV) The total number of failures was 5 cases (20 %) No therapeutic failures occurred among patients with acute tubular necrosis Three failures were in the form of a relapse with the same bacteriuria All these patients had microanatomical renal deformities I A phenacetin taker with bilateral deformity of the renal calices, II Left-sided renal papillary necrosis, III X-ray-opaque concrement in the left kidney The only absolute failures occurred in severely ill or debilitated glomerulonephritic patients one expired of staphylococcal sepsis and the other of his primary renal disease

The occurrence of secondary infection due to resistant organisms following the eradication of sensitive strains has been observed by various authors, e g Lubsen, in chronic urinary tract infections (19), and in cases of acute tubular necrosis by Liewendahl and Tallgren (17) The mechanism behind this phenomenon is poorly understood Fresh infection seems unlikely A sub clinical mixed bacteriuria probably existed prior to treatment This can sometimes be detected in stained smears but not in cultures The regularity with which the sensitive Klebsiella, pseudomonas and coli strains in this series were followed by *Proteus* and *Enterococcus* is striking In table III *Enterococcus* has proved poorly susceptible to polymyxin This is also partly true of *Proteus* Sub classifications of the proteus strains were not made This might have revealed a resistant sub species Middleton has reported the conversion of proteus infection into *Pseudomonas* during nitrofurantoin therapy (23) Synergism between colistin and sulphafurazole against *Proteus ssp in vitro* has recently been reported by Russell (25) This may be a pointer to the possible further development of colistin therapy Combining colistin with nitrofurantoin and/or sulphonamides in doses set according to renal function (27) might prevent secondary infection Enterococcus is easily eradicated with Na-benzylpenicillin This aspect was not systematically followed in this series, despite the utilization of combined therapy

The overall sensitivity of the bacterial species to polymyxin (table III) accords fairly well with other reports (8, 30) It also explains the nature of the secondary infections That colistin at therapeutic levels is inactive towards *Proteus ssp* has been reported by numer

ous authors (5, 28) The fairly high frequency of polymyxin B-sensitive *Proteus ssp* might be a consequence of the virtual absence of polymyxin treatment of systemic infections in our hospital prior to colistin

Analysis of the failures according to the bacterial species gives the following results The treatment of pseudomonas infections was in all cases successful Relapses with *E coli* occurred three times and with Klebsiella once This confirms the often expressed opinion that colistin is the drug of choice in pseudomonas and klebsiella infections

Nephrotoxicity

The possible nephrotoxicity of colistin treatment was observed by following urinary sediment proteinuria renal function and histology

Histopathological data are available in all but one case The histopathological findings do not differ however from the expected picture of the underlying disease Successive biopsies were not performed (except in one case) To differentiate an additional histopathological injury without comparison of the picture before and after assumed nephrotoxic exposure in a previously diseased kidney seems hardly possible Yow et al had renal biopsies in eight cases and Fekety et al had histology from three patients Neither could report any histological evidence of the nephrotoxicity of colistin (8 30)

Four patients had slightly increased proteinuria Only in the nephrotic patient could proteinuria be regarded as a sign of toxicity The changes in the urinary sediment could be accounted for by the underlying disease process

Renal function tests, such as endogenous creatinine clearance, phenosulphonephthalein excretion, concentration tests and ammonium chloride tolerance test for urinary H ion excretion, were performed too sporadically to permit of conclusive comparison The three latter tests are regarded as indices of tubular function The only significant changes that we have been able to record — within the scope of this series — showing a correlation to colistin treatment were variations in the plasma creatinine levels That plasma creatinine levels, with the only noteworthy exception of those in patients with atrophic muscular disease, show a good correlation with endogenous creatinine clearance has been demonstrated in numerous studies (6, 10, 15)

Increased serum urea nitrogen levels in response to colistin treatment have been observed by Fekety et al (8) This increase occurred only in patients with previously impaired renal function Out of a total of 24 patients he noted a transient mild elevation in 12 patients and a prolonged rise in 5 There was no permanent elevation

In our series a transient elevation of plasma creatinine occurred in acute reversible nephropathies Only three cases of acute renal failure had a transient increase above the initial base line However retardation of an expected fall during the polyuric phase occurred in four patients In three cases of acute renal failure no relative increase could be recorded Furthermore the rise in acute cases occurred within two days after treatment started Only the case of

TABLE VI Recommended dosage of colistin in chronic nephropathies with impaired renal function

Renal function	Normal	Mildly impaired	Moderately impaired	Considerably impaired	Gravely impaired
Plasma creatinine (mg/100 ml)	0 7—1 2	1 3—1 5	1 6—2 5	2 6—4 0	>4 0
Dose (I U)	3 000,000 thrice daily	3 500,000 twice daily	2,000,000 twice daily	3,000 000 once daily	3,500 000 —3 000,000 once every other day
Total daily dose (I U)	9,000,000	7,000,000	4,000,000	3,000 000	1,750 000 —1 500,000
Approximate daily dose/kg body weight (I U)	150,000	115,000	70,000	50 000	30,000 — 25 000

acute glomerulonephritis showed deterioration of the clinical course in connexion with the rising creatinine level In the acute cases the dose actually administered was not diminished according to the initial creatinine level (table I) We calculated that polyuric dilution of the excreted colistin load might reduce its nephrotoxicity in spite of still markedly elevated creatinine levels This assumption seems to have been erroneous in the case of the polyuric patient with acute glomerulonephritis (case 3)

We are inclined to recommend a colistin dosage of 2,000 000—4,000,000 I U daily in acute renal failure but not before the diuretic phase is well under way (daily urine output > 2,000 ml) Acute nephropathies other than acute tubular necrosis should not be included into this category

Chronic nephropathies seem to be more prone to prolonged or permanent deterioration of an already impaired renal function The relatively low doses recommended in this country by the manufacturer — 3,000,000 to 4,000,000 I U daily when renal function is normal — and the absence of complications even with mildly impaired renal function encouraged attempts with higher doses in highly resistant infections The dose administered was undoubtedly too large in some instances in this series Case 1 (fig 1) had received 6,000,000 I U daily for 9 days and case 2 (fig 2) 5,000,000 I U daily for 10 days with initial creatinine levels of 2 4 and 4 95 mg/100 ml respectively In chronic nephropathies the rise occurred somewhat more slowly in approximately 2—3 days

A permanent increase in the chronic cases is linked with a higher average dosage The seemingly paradoxical fall with an average dosage of 3,900,000 I U compared with the unchanged level at

3,200,000 I U must be attributed to the more effective antibacterial action without complicating nephrotoxic irritation This would imply that the optimal dose when renal function is moderately impaired is about 4,000 000 I U On the basis of our present experience and the studies of MacKay and Kaye (21), we recommend a colistin dosage as in table VI for chronic nephropathies with impaired renal function and sufficient urinary output

Summary

Colistin a compound apparently identical with polymyxin E, is a basic polypeptide antibiotic with potential nephrotoxic side effects Tissue damage at the site of injection is eliminated by use of its sulphomethyl derivative Colistin has an excellent antibacterial action *in vitro* upon *E coli Klebsiella* and *Pseudomonas* The drug is ineffective against *B proteus* and *Enterococcus* Synergism between sulphonamides and colistin might offer an extension of its spectrum to *B proteus*

A total of 25 patients with impaired renal function of varying aetiology were treated with colistin Only highly resistant urinary infections were selected for treatment In acute renal failure colistin was administered only in the diuretic phase Transient deterioration of renal function occurred in three patients with acute tubular necrosis Chronic nephropathies were more sensitive to higher doses showing signs of permanent renal damage In glomerulonephritic and nephrotic patients colistin might even be contra indicated

Dosage adjusted to the impairment of renal function is advocated for acute renal failures and chronic nephropathies Further studies are required, however, to establish the optimal dosage Dissimilar nephropathies seem to show different degrees of susceptibility Oliguria anuria is a relative contraindication Daily determinations of plasma creatinine levels are necessary in order to avoid injurious overdosage in the presence of impaired renal function When used with due caution, colistin is a valuable antibiotic in the treatment of resistant urinary infections caused by *Pseudomonas* and *Klebsiella* Coli infections can generally be treated successfully with less toxic antibiotics

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Acta Medica Scandinavica Vol 177, fasc 6, 1965

From Ullevaal Hospital Medical Dept VIII, Oslo, Norway

Perphenazin (Trilafon®) in the Prophylaxis of Nausea and Vomiting Following Acute Myocardial Infarct

By

Christopher Bjerkelund[1], Sigurd Nitter Hauge and Erling Jakobsen

Nausea and vomiting are frequently troublesome symptoms in the first stage of acute myocardial infarct They are variously reported as occurring in from 30 % to 60 % of patients (1 2 13 14), generally the more seriously ill Though primarily symptoms of the condition it self elicited through reflexes from the heart nausea and vomiting may be provoked by analgesics (particularly morphine) digitalis quinidine and other drugs as well

The vagus effects accompanying nausea and vomiting may include brady cardia vasodilatation and a resulting marked drop in blood pressure These effects may contribute to bring about post infarct circulatory collapse or they may worsen a pre existing state of shock or collapse

Since nausea and vomiting may thus influence the course and outcome of acute myocardial infarcts they merit more attention than has hitherto been given them especially since we now have a number of anti emetic drugs among which the phenothiazines are outstanding

Of these perphenazine (Trilafone®) seems the most promising In animals, its effect against apomorphine provoked vomiting is 16—24 times that of chlor promazine (10, 11) It is also effective against nausea and vomiting provoked by morphine, digitalis and other drugs

Clinically, perphenazine has been shown to combat vomiting following anesthesia or surgery as well as vomiting associated with gastro-intestinal diseases and pregnancy (3, 4 5, 6 7, 9 12)

In a controlled double blind study, we have investigated the effect of perphenazine on nausea and vomiting in acute myocardial infarct This has been done with special regard *also to the* effect of eventual prophylaxis against these symptoms on the course and outcome of *the infarct*

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Nausea and vomiting are frequently troublesome symptoms in the first stage of acute myocardial infarct They are variously reported as occurring in from 30 % to 60 % of patients (1, 2 13, 14) generally the more seriously ill Though primarily symptoms of the condition it self elicited through reflexes from the heart nausea and vomiting may be provoked by analgesics (particularly morphine), digitalis quinidine and other drugs as well

The vagus effects accompanying nausea and vomiting may include brady cardia vasodilatation and a resulting marked drop in blood pressure These effects may contribute to bring about post infarct circulatory collapse or they may worsen a pre existing state of shock or collapse

Since nausea and vomiting may thus influence the course and outcome of acute myocardial infarcts they merit more attention than has hitherto been given them especially since we now have a number of anti-emetic drugs, among which the phenothiazines are outstanding

Of these, perphenazine (Trilafone®) seems the most promising In animals its effect against apomorphine provoked vomiting is 16—24 times that of chlorpromazine (10, 11) It is also effective against nausea and vomiting provoked by morphine digitalis and other drugs

Clinically perphenazine has been shown to combat vomiting following anesthesia or surgery as well as vomiting associated with gastro-intestinal diseases and pregnancy (3 4 5 6, 7 9, 12)

In a controlled double blind study we have investigated the effect of perphenazine on nausea and vomiting in acute myocardial infarct This has been done with special regard also to the effect of eventual prophylaxis against these symptoms on the course and outcome of the infarct

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Methods

Since nausea and vomiting occur mainly in the first 48 hours following acute infarct, the observation time was limited to the first 32 hours after hospital admission

Included in the study were patients whose *condition* after the first clinical examination on admission was diagnosed as an acute myocardial infarct If more than 24 hours had elapsed since the acute attack, however, patients were included only if such acute symptoms as pain, dyspnea, shock, nausea or vomiting were still present

Perphenazine (Trilafon®) and the placebo (Nyegaard and Co) were packed separately in boxes of 3 ampoules, each box being intended for the treatment of one patient The boxes were randomised and numbered continuously by the manufacturer in such a way that equal quantities of placebo and perphenazine were included in a certain number of boxes in the test lot Each ampoule had the same number as the box containing it, and this number was placed on the chart of the respective patient

Thus none of the persons involved in the trial — investigators, administering physician, patient or nurse — knew whether the patient was given the placebo or perphenazine

Data were recorded carefully in two steps as follows

1 On selection for study each patient s name date of birth and assigned drug box number was recorded on his chart The time of the first injection was recorded and taken as the beginning of the observation period The times (date and hour) of the two subsequent injections were similarly recorded as were all occurrences of nausea and vomiting

2 The data on the chart as well as the nurse s comments and other materials from the case history were entered on individual summary-data sheets The following items were tabulated Name date of birth drug box number

Occurrence of nausea and vomiting a) before admission, b) after admission and before anti emetic therapy began Pulse temperature, blood pressure (twice daily over the first 48 hours after admission)

Occurrence of nausea and vomiting after commencement of anti-emetic therapy (a) and after each subsequent injection (b and c) a) between 0—8 hours, b) between 9—20 hours, c) between 21—32 hours

Occurrence during stay in ward of pulmonary edema, collapse or shock arrhythmia right heart failure and complications.

Dosages of concomitant medication

Thebaicine, morphine and meperidine were the only analgesics used If nausea and vomiting persisted in spite of treatment with perphenazine or the placebo, Dromyl® suppositories (diphenhydramine-8-chlortheophylline, 0 15 g) were given and the dosages recorded

Evaluation of the severity of nausea and vomiting was based on the reports of the nursing staff and particularly on repeated questioning and examination of the patients during the observation period This work was carried out by two of the investigating physicians each of whom evaluated nausea and vomiting in about one half of the patients over the period of the study The serial evaluations were always done by the same physician on the same patient Symptoms were graded or differentiated as 1) mild nausea 2) pronounced nausea, and 3) nausea and vomiting This judgement was of course subjective but since this was a double-blind study it seemed justified and was considered to be of some value

It was not considered possible to record the number of times vomiting occurred, or worth while to measure the total amount of material vomited The amount of vomit is in any case no indication of the gravity of vomiting in patients *with infarct*, since it depends primarily on how much the patient ate before the attack

In a controlled clinical trial it is worth knowing beforehand what comparisons are to be made as a basis for evaluating therapy Thus tables II—VI were designed before the study was begun and the *last column* (total number of cases) was completed before the examining physicians received instruc

TABLE I Patient distribution by sex and treatment group

	Perphenazine group		Placebo group		All patients		
	No	Average age	No	Average age	No	Average age	Age range
Men	48	57 9	39	60 0	87	58 7	37–84
Women	14	63 5	17	66 0	31	64 9	48–83
Men & women	62	59 0	56	61 8	118	60 4	37–84

TABLE II Presence or absence of nausea regardless of grade later than specified times from the start of anti-emetic therapy The figures in brackets indicate patients with nausea or vomiting before or on admission to the ward

Observation period	Perphenazine group	Placebo group	Total
1 1 hour			
No nausea	31 17	10 (2)	41 (19)
Nausea	21 16 40 4%	36 (20) 78 3%	57 (36) 58 2%
2 8 hours			
No nausea	38 23	12 4)	50 (27)
Nausea	14 10 26 9	34 (18) 73 9 %	48 (28) 49 0%
3 20 hours			
No nausea	46 27	26 (10	72 (37)
Nausea	6 6 11 5	20 (12 43 5 %	26 (18 26 5%

1 χ 12 88
P ~ 0 0003
2 χ = 19 73
P ≈ 0 00001
3 χ 11 19
P ≈ 0 0009

tions as to the allocation of perphenazin and the placebo

Perphenazine in doses of 3 5 mg each) or the placebo but not both was given in three intramuscular injections The first was given at admission the second and third eight and twenty hours later

Material

Originally 131 patients were recorded as suffering from acute myocardial infarct Diagnosis was not confirmed in 13 cases, leaving 118 patients for this study Distributions by sex age and drug are given in

Methods

Since nausea and vomiting occur mainly in the first 48 hours following acute infarct, the observation time was limited to the first 32 hours after hospital admission

Included in the study were patients whose condition after the first clinical examination on admission was diagnosed as an acute myocardial infarct If more than 24 hours had elapsed since the acute attack, however, patients were included only if such acute symptoms as pain, dyspnea shock nausea or vomiting were still present

Perphenazine (Trilafon®) and the placebo (Nyegaard and Co) were packed separately in boxes of 3 ampoules, each box being intended for the treatment of one patient The boxes were randomised and numbered continuously by the manufacturer in such a way that equal quantities of placebo and perphenazine were included in a certain number of boxes in the test lot Each ampoule had the same number as the box containing it, and this number was placed on the chart of the respective patient

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TABLE IV Effect of treatment on the presence or absence of nausea regardless of grade in each observation interval

Observation period	Perphenazine group	Placebo group	Total
1. 1—8 hours			
No nausea	35	16	51
Nausea	17 (32.7%)	30 (66.2%)	47 (48.0%)
2. 9—20 hours			
No nausea	41	13	54
Nausea	11 (21.2%)	33 (71.7%)	44 (44.9%)
3. 21—32 hours			
No nausea	46	26	72
Nausea	6 (11.5%)	20 (43.5%)	26 (26.5%)

1. $\chi^2 = 9.08$
   $P \approx 0.0027$
2. $\chi^2 = 23.24$
   $P \approx 0.00001$
3. $\chi^2 = 11.19$
   $P \approx 0.0009$

TABLE V Effect of treatment on the presence or absence of vomiting (in addition to nausea) in each observation interval

Observation period	Perphenazine group	Placebo group	Total
1. 1—8 hours			
No nausea and vomiting	44	19	63
Nausea and vomiting	8 (15.3%)	27 (58.7%)	35 (35.7%)
2. 9—20 hours			
No nausea and vomiting	46	23	69
Nausea and vomiting	6 (11.5%)	23 (50.0%)	29 (29.6%)
3. 21—32 hours			
No nausea and vomiting	48	36	84
Nausea and vomiting	4 (7.7%)	10 (21.7%)	14 (14.3%)

1. $\chi^2 = 18.10$
   $P \approx 0.00002$
2. $\chi^2 = 15.53$
   $P \approx 0.00008$
3. $\chi^2 = 2.87$
   $P \approx 0.09$

TABLE III Presence or absence of vomiting (in addition to nausea) later than specified times from the start of anti emetic therapy

Observation period	Perphenazine group	Placebo group	Total
1 1 hour			
No vomiting	41	15	56
Vomiting	11 (21 2%)	31 (67 4%)	42 (42 9%)
2 8 hours			
No vomiting	43	21	64
Vomiting	9 (17 3%)	25 (54 4%)	34 (34 7%)
3 20 hours			
No vomiting	48	36	84
Vomiting	4 (7 7%)	10 (21 7%)	14 (14 3%)

1 $\chi = 19\,46$
$P \approx 0\,00001$
2 $\chi^2 = 13\,19$
$P \approx 0\,0003$
3 $\chi^2 = 2\,82$
$P \approx 0\,09$

table I Both average ages and age range are recorded

Certain problems in the tabulation of data

1 *Mortality* Of the 118 patients, twenty died so soon that anti emetic effects could not be evaluated Half of these had received perphenazine and the others, the placebo

Twenty more patients died subsequently and were also evenly divided between the perphenazine and the placebo group Thus mortality in the perphenazine group was 32 % and in the placebo group 36 % The difference is not significant

2 *Evaluating anti emetic effect* Of the 98 patients followed during the whole observation period 52 received perphenazine and 46 received the placebo

In order to evaluate the effects of the drug on nausea or vomiting we tabulated the following two sets of data

a Data showing in how many patients these symptoms were still present later than specified times since the start of the drug In other words, how long the symptoms persisted in spite of the therapy (tables II and III)

b Data comparing the amount of symptoms in the placebo group and the perphenazine group for *separate time intervals* (tables IV and V) This information was needed because it was possible that some patients might not have developed nausea and vomiting until later periods during the observation

In compiling the above data account was taken of the fact that intramuscular perphenazine requires 30 to 60 minutes to achieve its full effect Hence in tables II—V we did not include symptoms occurring within an hour after the first injection in our calculations

Results

A comparison of the incidence of nausea (without regard to severity) in both groups (table II) shows that about twice

TABLE VII Average consumption of analgesics (morphine thebaine and meperidine) Milliliters per patient at serial times during observation period

Observation period	Perphenazine group		Placebo group	
	Analgesics (ml)	S E of mean	Analgesics (ml)	S E of mean
On admission	0 69	± 0 07	0 89	± 0 07
1—8 hours	0 41	± 0 09	0 74	± 0 10
9—20 hours	0 36	± 0 09	0 75	± 0 04
21—32 hours	0 31	± 0 08	0 57	± 0 04

The figures speak for themselves, but χ^2 and probability values are provided for orientation

Further comparisons

The contributory anti emetic effect of Dromyl suppositories remains to be discussed since these were given when perphenazine or placebo-treated patients continued to experience troublesome nausea and vomiting Dromyl was required by eighteen patients in the placebo group but by only two in the perphenazine group (table VI) The difference is statistically significant and supports the previous results in this study

If Dromyl had an anti emetic effect in these cases it should have reduced the gross difference between the two groups in the incidence of nausea and vomiting But since Dromyl was not given till eight hours had elapsed this difference would be manifest only in the last two time intervals

Table VII gives the average use of analgesics on admission and in the subsequent three intervals The perphenazine group needed much less than the placebo group It will also be noted that eight patients in the perphenazine group required no analgesics after admission in contrast to one patient in the placebo group We may assume that perphenazine had a potentiating or sedative effect which reduced the need for analgesics

As previously mentioned, analgesics may provoke nausea and vomiting in some patients Thus, it is possible that perphenazine reduced these symptoms not only by direct anti emetic action but also by reducing the need for analgesics

Effect of perphenazine on the course of the infarct

It was assumed that this effect would show itself in blood pressure measurements as well as in the amount of pulmonary edema, collapse, shock, arrhythmia and signs of right heart failure during treatment on the ward

Table VI shows that one or more of these complications occurred more often in the placebo group than in the perphenazine group and that pulmonary edema and cardiac arrhythmia occurred in a 2 1 ratio The numbers are small

TABLE VI Patients who had pulmonary edema, collapse or shock, arrhythmia, right heart failure, or one or more of these four complications Patients treated with digitalis, oxygen or Dromyl suppositories

		Perphenazine group		Placebo group	
		No	%	No	%
Complications					
Pulmonary edema	(1)	3	5 8	6	13 0
Collapse or shock	(2)	5	9 6	7	15 2
Arrhythmia	(3)	7	13 5	13	28 3
Right heart failure	(4)	5	9 6	6	13 0
One or more of 1, 2, 3 or 4		15	28 8	23	50 0
Treatment with					
Digitalis		8	15 4	10	21 7
Oxygen		11	21 2	15	32 6
Dromyl suppositories		2	3 9	18	39 1

as many placebo patients (78 3 %) as perphenazine patients (40 4 %) experienced nausea This difference is statistically significant at the 1 % level ($\chi^2 = 12\ 88$) Towards the end of the observation period (after 20 hours) there was almost four times as much nausea in the placebo group (43 5 %) as in the perphenazine group (11 5 %) This difference is also statistically significant at the 1 % level ($\chi^2 = 11\ 19$)

The bracketed figures in table II show how many patients experienced nausea before or on admission A comparison of these figures with the later figures in the table shows that perphenazine reduced nausea in those who were already nauseated and prevented the occurrence of nausea during the observation period

A comparison of all cases of vomiting in addition to nausea (table III) shows that during the observation period the placebo group had three times as high an incidence of these symptoms (67 4 %) as the perphenazine group (21 2 %) The same proportions were obtained if we compared the number of cases with vomiting after 20 hours 21 7 % to 7 7 %

Similar proportions were also obtained by tabulating the cases of severe nausea and vomiting in the same way

Tables IV and V compare, respectively nausea regardless of grade and vomiting in addition to nausea, in the separate time intervals following injections (1—8 hours, 9—20 hours and 21—32 hours) Here, too, the effect of perphenazine is clearly in evidence It can be seen that both the raw figures and the percentages stand in about the same proportions as the figures in tables II and III Thus, most patients with nausea and vomiting in one of the later intervals had also had troubles in the preceding interval

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and not statistically significant, but the bias is on the side of the perphenazine group It is not possible to decide whether this bias is an effect of the perphenazine on nausea and vomiting, or whether it is a result of the randomising procedure, i e purely accidental A considerably greater number of cases would be required to settle this question

We have already mentioned that there was no significant difference in the incidence of deaths between the two groups Nor did systolic and diastolic blood-pressure variation in the first two days after admission show any significant difference

No extrapyramidal or other side effects were found, possibly because of the low dosages given

Summary

The effect on acute myocardial infarct patients of treatment with perphenazine was observed in a carefully planned, controlled, double blind study

Factors for comparison and techniques of evaluation were outlined before commencement of the study

The case material comprised 118 patients with infarct, 62 of whom were given perphenazine and 56 of whom were given the placebo Twenty patients died too early in the study to be evaluated for the effect of perphenazine Of the remaining 98 patients, 52 were given perphenazine and 46 the placebo

The study showed a marked reduction in the incidence of nausea and vomiting in the perphenazine group as against the placebo group This difference was statistically significant at the 1 % level

Perphenazine both reduced nausea and vomiting initially present and prevented their later appearance

Another anti-emetic (Dromyl), which was given if nausea and vomiting persisted, was used mainly in the placebo group

Perphenazine also reduced the need for analgesics Since analgesics may provoke nausea and vomiting in some patients, it is possible that perphenazine reduced these symptoms not only by a direct anti emetic action, but also by reducing the need for analgesics

While perphenazine treated patients showed fewer complications, especially pulmonary edema and cardiac arrhythmia, the differences were not statistically significant

No significant differences in mortality during the stay in hospital were found

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From Medical Service I (Head Lars Werko M D) Sahlgren s Hospital, University of Goteborg Sweden

A General Health-examination of a Random Sample of 50-year-old Men in Göteborg

By

Gosta Tibblin, Elisabeth Aurell, Hans Hjortzberg-Nordlund Sven Paulin, Lars Risholm, Harald Sanne, Lars Wilhelmsen and Lars Werkö

Can one trust the Swedish saying each pain has its cry" or are health examinations necessary to reveal silent diseases? Several general health examinations have been started in Sweden in recent years which suggests there is already acceptance of the value of health examination for the detection of disease It is clear from a recent review (1) of national and foreign examinations in this field that the scientific arguments for the value of large scale health examinations are founded *merely* on assumptions

In the examinations so far reported the central aim has been to diagnose as many various conditions as possible by an essentially standard technique (questionnaire and to a varying extent physical examinations as well as various laboratory tests — from simple routine tests to chemical health controls) The precision of the examination has been checked only incidentally How many examined cases remain undiagnosed? How large will the gain of revealed cases be, if the examination is extended with more investigations, with more laboratory tests or with both? Is it possible to influence the future morbidity of a group through a general health examination?

During 1963 a combined health examination and population study of 973 men, all of whom were born in 1913, was performed at the Sahlgren s Hospital in Goteborg It was a 4 hour study of the individual s state of health with use of the personnel and technical resources of a hospital

The *object* of the *examination* was partly to diagnose the participants unknown diseases and partly to evaluate the separate *diagnostic methods* A *follow up* of the participants after the examination was planned so as to evaluate to what *extent the health*-examination had managed to find those with early or manifest diseases This made it possible to study how the future morbidity of the examined group had been *influenced*

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TABLE I Laboratory examinations

Chemical constituents of serum
Electrophoretic fractions of proteins
Cholesterol
Glyceride Glycerol
Glucose
Creatinine
Sodium
Potassium
Chlorides
Haematocrit
Blood groups (ABO, MN, Duffy, Kelly, Rh)
ESR
Urine
Glucose
Protein
Osmolality

The population study was partly a study of the frequency of certain diseases, partly an examination of variables which have been connected with coronary disease The result of the population study will not be discussed in this report

Methods

The planning of the population study made it desirable only to examine men of the same age The chosen age group should consist of men born in 1913 The selection was done by the Goteborg Revenue Office All men who were living in Goteborg and who were born on a day the date of which was a multiple of three were included in the material In this way 973 men were selected These were invited by letter to a health-examination

Every week-day morning 4—6 participants came for examination They arrived at 7 15 and were then fasting and in most cases also thirsting During the first 45 minutes they passed four stations At the first station blood and urine samples were taken Three separate portions each of 3 ml were stored in a deep freeze. The chemical laboratory tests used in the examination are listed in table I At the second station E C G was recorded The tracings were classified according to the Minnesota Code At the next station the same doctor interviewed all subjects about work, social background chronic bronchitis and angina pectoris In the medical history the questionnaire on cardiac pain recommended by WHO 1962 and a questionnaire approved by the British Medical Research Council's Committee on the Aetiology of Chronic Bronchitis were used At the last station anthropometric measurements were made according to Lindegård At about 8 o'clock an X ray of the heart and the lungs was taken which included heart volume estimation both in supine and standing position

After a light breakfast — which was taken with some of the investigators — all participants took part in an interview about previous diseases, whereupon they were subjected to a physical examination, measurement of blood pressure an examination per rectum and an estimation of peak flow according to Wright The blood pressure was measured after a 5—10 minutes talk with the participant The pressure was measured on the subject's right arm using a sphygmomanometer with a rubber bag, which was 12 cm wide and 23 cm long The systolic and the two end-diastolic pressures were registered both in sitting and standing positions

The participants who were born on an odd as distinct from an even date had a psychiatric interview the main object of which was to find out the individual's power of resistance to strain, hurry excitement and similar situations Those born on an even date went through an examination of the lung function where vital capacity, FEV 1 0 and a measure of the degree of obstruction are determined Those born on an even date also had to answer a questionnaire dealing mainly with the same problems that were taken up in the psychiatric interview

At about 10 o clock the participants went to the department of ophthalmology where the ophthalmologist of the team made an examination of the eyes with special regard

to the vessels of the retina A photograph of the fundus was also taken

One year in advance records and X ray reports had been collected For about 60 % of the participants it was possible to get information from the physical examination carried out before military service (at the age of 20) with regard to height weight and also blood group One month before the day of examination the participants received an information letter One week before, they received a notification letter The evening before, a secretary called the participants by telephone and gave them further information A few days after the examination the first summing up was done It was decided whether any measures were required The acute cases were hospitalized The members of the medical staff themselves when necessary served as consultants For further investigation a special clinic session was arranged which was held every evening Most of the checking of divergent laboratory tests was done then and the surgical conditions considered to require treatment were also taken care of The laboratory values that automatically led to further investigation were haematocrit < 40 % SR ≥ 20 mm plasma creatinine ≥ 1 5 mg % and the occurrence of glucosuria as well as proteinuria A diastolic blood pressure of ≥ 110 mm Hg – hypertensive changes of the eye ground also led to investigation About three weeks after the examination a summary of each case was written If the participant had his own physician the latter received a copy The participants got a brief account in which a telephone time was given in case any of them wanted further information regarding the examination they had received

No public announcement in the press radio or TV preceded the health-examination It was considered necessary to take some step to foster motivation to participate in the examination The Trade Union Movement the Employers General Association the City of Göteborg and the industries of the city were contacted All the consulted parties approved of the idea of the examination and during its progress they gave it remarkable

TABLE II The number examined at the hospital or at home, among 973 50 year-old men (The Goteborg-examination 1963)

	No	%
Examined at the Sahlgren s Hospital, Goteborg	855	88
Examined at home	40	4
Not examined	78	8
Total	973	100

TABLE III Internal medical conditions leading to hospitalizing or treatment in the out patient ward (The Goteborg examination 1963)

Diagnoses of the hospitalized participants	No
Pulmonary tuberculosis?	2
Hypertensive disease	8
Iron-deficiency anaemia	1
Haemolytic anaemia	1
Auricular fibrillation	2
Malabsorption	1
Total	15
Diagnoses of participants treated in the out patient ward	
Hypertensive disease	8
Iron-deficiency anaemia	8
Angina pectoris	2
Diabetes mellitus	2
Renal glycosuria	1
Congestive heart failure	1
Congenital heart disease?	1
Paroxysmal auricular tachycardia	2
Obesity	3
Primary essential xanthomatosis	1
Paraproteinaemia	2
Degenerative joint disease	2
Proteinuria	1
Remaining diagnoses	5
Total	39

TABLE IV Surgical conditions leading to hospitalizing or treatment in the out patient ward (The Goteborg examination 1963)

Diagnoses of hospitalized participants	No
Varicose veins	6
Inguinal hernia	7
Polyp of rectum	2
Haemorrhoids	2
Varicocele of spermatic cord	1
Examination of the urinary tract	1
Total	19
Diagnoses of participants treated in the out patient ward	
Haemorrhoids	15
Varicose veins	4
Prostatitis	3
Polyp of rectum	2
Contracture of Dupuytren	2
Hydrocele	1
Nephrolithiasis	1
Haematuria	1
Dumping syndrome	1
Diaphragmatic hernia	1
Low back pain	2
Post thrombotic syndrome	1
Total	34

TABLE V Remaining conditions leading to hospitalizing or treatment in the out patient ward (The Goteborg-examination (1963)

	Hospitalized	Treated in out patient ward
Psychiatric conditions	2	10
Otologic conditions		
Deafness		4
Sinusitis		1
Pharyngitis		1
Tumor labiale	1	
Leucoplakia	1	
Dermatologic conditions		
Candidioma		1
Neurodermatitis		1
Rosasea c acne		1
Verruca vulgaris		1
Eczema		1
Ophthalmologic conditions		
Optic atrophy	1	
Glaucoma simplex	1	1
Total	6	22

support, thus many of the participants could attend the examination without loss of income

Results

During 1963, 855 participants (table II) were examined at the Sahlgren s Hospital in Goteborg One of the physicians of the group made calls at the homes of 40 of the participants who had refused to come to the hospital, and interviewed them regarding their state of health 78 men (8 %) did not participate in any kind of investigation The loss of participants will be analysed separately

The health-examination findings caused 36 participants to be hospitalized At the special investigation in the out patient clinic 95 participants were diagnosed and received treatment — mainly for conditions belonging to the surgical and internal medicine specialities The number of participants where illness was suspected but control examination gave a negative result, amounted to 35

Among the conditions belonging to internal medicine hypertensive disease

TABLE VI The contribution of the different examination methods towards the diagnosis made (The Göteborg-examination 1963)

Specialty	History + physical examination	Chemical laboratory tests	ECG	X ray of heart and lungs
Internal medicine	30	18	2	4
Surgery	51	1	0	1
Psychiatry	12	0	0	0
Otology	8	0	0	0
Dermatology	5	0	0	0
Ophthalmology	3	0	0	0
Total	109	19	2	5

(16 participants) and anaemia (10 participants) were most common (table III) Of the anaemia cases 5 had been operated on for peptic ulcer

The surgical conditions were dominated by varices, varicose veins, inguinal hernia and haemorrhoids (table IV) The remaining conditions that led to hospitalization or out patient treatment are listed in table V

The eye diseases should be specially noted The ophthalmologic examination comprised determination of refraction tonometry and ophthalmoscopy About 15 % of the participants appeared to be in need of lens correction in most cases for use when reading The majority of them got a prescription for new spectacles By repeated examinations an increased intraocular pressure (≤ 6 0,7 5 g) was found among 16 participants In all cases this increase of the eye pressure had not hitherto been discovered Two patients were put under continuous pressure reducing treatment The other 14 had only a moderate increase of pressure (6 0—5 9 7 5 g) and during the observation period of 1—1 1/2 years they have not shown any tendency towards a further increase Two participants were hospitalized for investigation One patient had glaucoma simplex and the other case a unilateral optic atrophy with a history of reduced vision since half a year In spite of a thorough ophthalmo-neuro-radiologic investigation it was not possible to give any explanation of the atrophy of the optic nerve in this case

No cases of cancer were found On the other hand 6 cases of pre malignant conditions were found four of which were rectal polyps

In order to get an idea as to what part of the examination led to diagnosis it was divided into 4 parts history + physical examination chemical laboratory tests X ray and electrocardiogram (table VI) Each diagnosis was referred to only one method of examination History + physical examination proved to be superior to the other methods for revealing disease conditions among these middle aged men With the aid of this method 81 % of the noted diagnoses

TABLE VII Deaths, diagnoses and course of disease among participants taken ill 8–20 months after the examination, with vascular diseases or malignant conditions (The Göteborg-examination 1963)

Diagnoses	Symptoms at the examination	First discovery of symptom (time after exam)	Course of disease
1 Myocardial infarction	0	3 months	+ 3 months after ex
2 Myocardial infarction	0	14 months	Hospitalized 14 months after ex
3 Myocardial infarction	0	14 months	Hospitalized 14 months after ex
4 Myocardial infarction (?)	0	15 months	Hospitalized 15 months after ex
Hemiplegia dx cum aphasia	+	1 week	Hospitalized 1 week after ex
Tumor cerebri	0	2 months	+ 12 months after ex
Cancer of tongue	0	2 months	Hospitalized 4 months after ex
Papilloma of bladder	0	9 months	Hospitalized 9 months after ex
Chronic myelocytic leukaemia	0	10 months	Hospitalized 10 months after ex
Bronchial asthma	+	—	+ 2 months after ex
Veneficium	0	5 months	+ 5 months after ex
Aneurysma of a pericallosa dx	0	8 months	+ 9 months after ex

were determined The laboratory tests revealed 14 % of the diagnoses

In 35 participants repeated examination did not bear out the suspicion of illness The reason for the second examination was in 22 cases the divergence in the laboratory results

Short term follow-up

In order to analyse the influence of the health-examination on the future mortality and morbidity of the examined group, the death certificates of this year group were checked 1 9 1964 It was then found that 5 of the examined participants had died At the same time an inventory was made of the records at the hospitals of the City to ascertain whether any of the participants had been hospitalized for a reason other than the diseases found at the health-examination The period of observation was from the day of the health-examination to the checking date between 8 and 20 months later The records of those who had died or had been taken ill with vascular diseases or malignant conditions were subjected to closer analysis From table VII the diagnosis of the disease and the occurrence of symptoms at the time of the health examination can be seen as well as the length of the period from the health examination to the time of the appearance of the disease The symptoms at the health examination have been recorded as well as physical or chemical signs of the approaching disease (high sedimentation rate, low haemoglobin content, high blood pressure etc) Only 2 participants showed any symptoms or

TABLE VIII Lip d -alues blood p essure he ght and we ght f om the health exam nat on of 1963 of the partic pants la er developing myocardial infarction (The Gotebo g-examinat on 1963)

	Mean for the ent re material and S D	No 1	No 2	No 3	No 4
Chol s erol (mg/100 ml)	247±43	322	223	259	191
Gl cer de Glycerol (mMol/l)	1 26± 0 82	0 97	1 17	1 03	0 94
Systolic blood p essure (mm Hg)	138±21	120	105	150	135
Dias olic blood pressure (mm Hg)	91±15	80	70	105	95
He ght (cm)		169	176	176	178
We ght (kg		71	71	83	94

s gns at the t me of the health exam na tion One such case was a man w th bronch al asthma who was already un der med cal treatment In the other pat ent serious hypertension was found at the health-exam nat on leading to immed ate hosp tal zat on The vascular acc dent occurred before treatment was started Preventive measures have been espec ally discussed in connect on w th coronary heart d sease Reduction of body we gl t treatment of h gh blood pressure and alteration of blood l p ds with d et or the use of drugs have each been suggested It is therefore of nterest tl at among the small group of myocar d al infarct ons occurring after the ex am nat on no one l ad hypertens on or was o er e gl t Nor was there any reason to correct tl e r blood l p ds (table VIII)

Bes des tl is group of part c pants who were taken ill w th serious cond t ons there were 38 pat ents who for otl er reasons were l o p tal zed Among these attent on should be dra n to a group of 11 w l o rece ved med cal care on account of acc dents Tw o pat ents had commotio cerebri and one case contus o cerebri Cholel thiasis and pept c ulcer with or without haemorrhage were other fre quent causes for hospitalization (12 pa tients) Not even frequent health exam inat ons can prevent such accidents or acute abdom nal diseases There has thus been no reason for a more thorough study

Discussion

The number of new d agno es has been the measure of the ab l ty of the health exam nation to reveal diseases For a ne vly discovered d agnos s to be counted it was requ red that t should lead to further in est gat on In mo t cases th s also meant treatment Accordingly par t c pants w th for nstance non specific ECG changes without *symptoms over* we ght or d vergent laboratory values w thout cl n cal connect on were not d agnosed as being ill The reason for this lim tat on was the wi h to bring out only those cond t ons for wh ch som kind of treatment could be offered Another reason was that f criteria of disease were expanded also to nclude for instance

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in the fight against chronic diseases It should not, however, be used to enlarge the concept of disease to comprise everybody exhibiting such an abnormality The biological meaning of such results may still be obscure because the predictive value has a statistical meaning only in a specified population and helps to describe the evolution of disease in a group, not on an individual basis In earlier general health examinations it has been found that only a small number of those examined were 'healthy The 1954 health examination in Stockholm comprising 2,504 subjects (2) gave 4,636 diagnoses which is almost two diagnoses per participant Similarly Robert (7) found that of the male adults who believed themselves to be healthy' only 10—25 % in fact were completely healthy Another American examination (8) revealed that of 10,709 apparently healthy persons 92 % had diseases or defects of varying degrees Thus the general health-examinations have been used as a measurement of the general morbidity of an examined group Attention has not been paid to the importance of the methods used to get a correct conception of a disease Special measuring instruments are required for each disease entity to be studied In order to investigate the prevalence of high blood pressure (4) standardized conditions in the examination and specially trained personnel are required The prevalence of cancer among a population can be found only through the use of an advanced technique The diagnosis of uterine cancer required cytologic experts and the diagnosis of carcinoma of breast well trained examiners Studies of the frequency of rheumatoid arthritis eye diseases and skin diseases also require their own special technique (3) These important methodological problems have only seldom been discussed The resulting picture of the morbidity is on account of this marred by large errors One single examination, moreover can never give a correct picture of all diseases among one group of examined people Instead one must be content to study only one or a few diseases by the technique specially designed for this purpose This can with advantage be done within the limits of a general health examination

In the present examination 36 participants were hospitalized as a result of the study At the follow up investigation in the out patient department a further 95 patients were found to have some specific disease The reason why every 7th participant had an illness not earlier diagnosed might be either that the disease in question had not shown particularly troublesome symptoms or that the participants were not conscious of any symptoms The group of subjects not acquainted with the presence of illness consisted of 41 participants Thus it seems that with the above method one has to examine 20 middle aged men to find one who is unconscious of his illness and who is in need of treatment The dominating conditions were hypertensive disease and *anaemia (26 cases)*

Only a small number of those that had died or were taken ill with malignant or vascular diseases when the group was followed up 8—20 months later showed symptoms at the time of examination This demonstrates that in spite of a

divergent laboratory values the result would be that most of the participants would receive a diagnosis only because of the diversity of the examined variables

In general health examinations as well as in studies of the aetiology of diseases, the delimitation of various diseases is one of the main problems It is necessary to get as homogeneous a material within a disease as possible, while the different disease entities ought to be as divergent as possible among themselves The development of the medical sciences and especially the introduction of many new laboratory techniques has changed our concepts of disease entities Whereas the clinical picture of the patient was once the most important clue to his illness, nowadays a tendency has appeared to use laboratory values of more or less pronounced specificity to describe different diseases This is due partly to increasing knowledge — for example that Muller-Harbitz disease, xanthomatosis, has high blood lipid values as a part of the picture and often leads to myocardial infarction — and partly it is caused by increased interest among the general population as well as among physicians in the prevention of diseases, leading to an extensive use of laboratory tests in symptom-free individuals The evaluation of a single — or several — laboratory results deviating from the established norm, poses a new and difficult problem to solve By some it has been solved simply by defining the disease entity with the use of abnormal laboratory values — latent diabetes when the blood sugar is slightly elevated essential hypercholesterolaemia when the blood cholesterol exceeds an arbitrarily selected limit of 260, 280 or 300 mg per 100 ml blood, as the investigator wished (the limit selected reflecting how important the investigator considers his laboratory tests to be for the future development of disease) In a similar way diseases like obesity, anaemia and hypertension are constructed It is obvious that these disease entities within themselves are heterogeneous and, moreover, are difficult to delimit from other categories Murphy (6) has pointed out that only with a small number of variables can a classification be made into separate disease groups Thus on the whole it is impossible only with the help of a quantitative variable to delimit a part of the population with one specific disease From this it follows that concepts of diseases such as essential hypercholesterolaemia, obesity, or essential hypertension founded merely on cholesterol values, weighing and measuring of blood pressure do not have any justification, either when results from a health examination are presented or when the aetiology of diseases is studied

This negative attitude towards the use of laboratory values for the description and delimitation of diseases should not be interpreted as a general denunciation of the value of laboratory tests in population studies On the contrary it is important to study the frequency distribution of the results of several such tests in the healthy population in order to define their predictive value regarding the development of future overt diseases If a predictive value of some such test could be established — which so far does not seem to be the case notwithstanding many reports maintaining this — this would represent an important advance

in the fight against chronic diseases It should not however, be used to enlarge the concept of disease to comprise everybody exhibiting such an abnormality The biological meaning of such results may still be obscure, because the predictive value has a statistical meaning only in a specified population and helps to describe the evolution of disease in a group, not on an individual basis In earlier general health examinations it has been found that only a small number of those examined were healthy' The 1954 health examination in Stockholm comprising 2,304 subjects (2) gave 4,636 diagnoses which is almost two diagnoses per participant Similarly Robert (7) found that of the male adults who believed themselves to be "healthy' only 10–25 % in fact were completely healthy Another American examination (8) revealed that of 10 709 apparently healthy persons, 92 % had diseases or defects of varying degrees Thus the general health examinations have been used as a measurement of the general morbidity of an examined group Attention has not been paid to the importance of the methods used to get a correct conception of a disease Special measuring instruments are required for each disease entity to be studied In order to investigate the prevalence of high blood pressure (4), standardized conditions in the examination and specially trained personnel are required The prevalence of cancer among a population can be found only through the use of an advanced technique The diagnosis of uterine cancer required cytologic experts and the diagnosis of carcinoma of breast well trained examiners Studies of the frequency of rheumatoid arthritis, eye diseases and skin diseases also require their own special technique (3) These important methodological problems have only seldom been discussed The resulting picture of the morbidity is on account of this marred by large errors One single examination, moreover, can never give a correct picture of all diseases among one group of examined people Instead one must be content to study only one or a few diseases by the technique specially designed for this purpose This can with advantage be done within the limits of a general health examination

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Only a small number of those that had died or were taken ill with malignant or vascular diseases when the group was followed up 8—20 months later showed symptoms at the time of examination This demonstrates that in spite of a

careful examination a relatively large group of men are taken ill or die within about a year's time of examination irrespective of the health-examination In the small group of later myocardial in farction no preventive measures were indicated at the time of examination

In the present investigation the extent of the population-study justified a thorough and time consuming examination There is no indication that such a detailed health examination adds much useful knowledge At the ophthalmological examination, for instance, 16 cases with increased intraocular pressure were found, but only two of those demanded treatment At earlier mass examinations in Skövde (5), a group was revealed with moderately increased intraocular pressure corresponding to the above-mentioned cases These subjects have been followed for 3 years During this period there have not been any signs of progress The delimitation of the disease glaucoma cannot simply be made with only one isolated measurement of the pressure

The result of this investigation indicates that general health examinations can, to a limited extent, contribute to reveal important silent" diseases These general health examinations have many draw-backs (9) They are expensive and time consuming for the participant as well as for the examining personnel It is necessary to decide quickly whether a person is ill or healthy, which may be a difficult question A general health examination may jeopardize an important personal relationship already established between patient and physician A better alternative to the general health-examination is an examination directed against specific diseases The measurement of blood pressure and haemoglobin appears to be a sufficient examination for the middle aged men, in order to find the most important and most common of the "silent" diseases The technical apparatus for such an examination is insignificant A screening process can to a large extent be done with the help of an unqualified staff Such examinations may for instance be made at the place of work Since anaemia and hypertension are as important problems for women as they are for men, both sexes may be examined simultaneously These two conditions are well known to the public The therapeutic measures indicated will therefore have a fair chance of meeting with a general response by those participants who are found to have the disease in question

It is uncertain whether the introduction of general health examinations into the present community will change the extent, distribution and prognosis of diseases, especially if the examinations are not repeated at rather short intervals in the same group Such a change will be difficult to define, not only because the registration and evaluation of the extent of illness in one community are rather imperfect but also because other advances in the diagnosis and treatment of many diseases will confuse the picture The promotors or proponents of the idea of general preventive health examinations, who seldom belong to the active medical field or at least do not work in clinical or epidemiological sciences, will thus have good opportunities to continue the present propaganda founded on theories rather than on prac-

tical experience and well controlled results

On the other hand, if, all of a sudden there were a halt in the consumption of tobacco nicotine, and excess calories especially in the form of refined sugar and animal fat, the general health of the population would undoubtedly be markedly improved If this happened simultaneously with a general increase in the time spent in pleasant forms of physical exercise and in extra restful sleep, the distribution and course of diseases would undoubtedly change as markedly as after the introduction of antibiotics These are, however, unrealistic and utopian proposals

The conclusion from the health examinations of 1954 in Stockholm was that directed health examinations and health propaganda combined are the measures that seem to be most useful to the community and also feasible to conduct Ten years have passed since then and this thesis is still valid

Summary

An extensive study of the health condition of 855 50 year old men showed that 5 % had diseases unknown to the examined and demanding treatment Anaemia and arterial hypertension were the most usual conditions revealed The draw backs of a general health-examination are discussed and a proposition is made recommending solely examinations directed towards these two diseases for this age group of men

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From the Department of Pediatrics (Head S Axtrup, M D), Central County Hospital Kristianstad Sweden and from the Department of Internal Diseases (Head J Waldenstrom M D) Malmö General Hospital, Malmo Sweden

Hereditary Hypergammaglobulinemia and Lupoid Hepatitis

By

BERTIL CAVELL and TORE LEONHARDT

Waldenstrom (17) and Kunkel et al (7) described a special form of chronic hepatitis with a predilection for young women Characteristic of this disease is its self perpetuating course marked hypergammaglobulinemia, infiltration of plasma cells and lymphocytes in the liver, and usually ready response to adrenocorticosteroid therapy Mackay et al (11) showed that some patients with this form of hepatitis also had a positive LE cell phenomenon and systemic manifestations suggestive of systemic lupus erythematosus (SLE) They called the syndrome lupoid hepatitis a term which is now in common use According to Mackay et al immunologic destruction of liver cells rendered antigenic by viral or possibly nutritional damage best explains the perpetuation of the hepatitis and its progression to cirrhosis and excess production of abnormal antibodies might explain the other features of the syndrome such as arthralgia hemolytic anemia and LE cell phenomenon

Leonhardt (9) described the occurrence of SLE in 3 of 14 siblings, most of whom had hypergammaglobulinemia An inherited tendency to overproduction of gammaglobulin with consequent disposition for SLE was postulated The assumption of a genetically determined overproduction of gammaglobulin was strengthened by examination of further members of this family and of the relatives of another proband with SLE (8) Later on Leonhardt (10) reported on the occurrence of hypergammaglobulinemia rheumatoid factors antinuclear factors and other serologic factors in the pedigrees of 109 probands with definite or suspected SLE That study confirmed the earlier impressions that genetic mechanisms are involved in the causation of SLE

In view of the similarities between lupoid hepatitis and SLE and of the familial background of hypergammaglobulinemia and other serologic abnormalities in the latter it was decided to study the occurrence of such abnormalities

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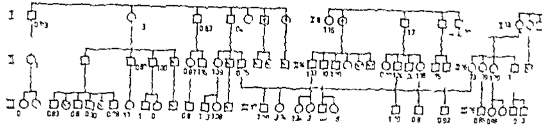


Fig. 1. Pedigree of proband with lupoid hepatitis. † points to the proband. The age-corrected gammaglobulin values are given for all individuals tested. NT = not tested.

in the relatives of a patient with lupoid hepatitis.

Material and methods

The case report of the *proband*, an 11 year-old girl with lupoid hepatitis, is given below.

Twenty-three paternal and 31 maternal *relatives* of the proband were examined. A few of the living members of the pedigree were not included in the material either because they lived too far away or because they were unwilling to co-operate (fig. 1).

Twenty married partners of the proband's relatives were included as *controls*. Because of the smallness of the sample, however, it was decided to use another control material as a reference, namely 324 married partners of patients with SLE and of their relatives included in the investigation of Leonhardt (10). This latter investigation had been carried out according to the same principles as the present study.

The sex and age distribution of the groups of individuals in the study is given in table I.

The concentration of serum gammaglobulin was calculated from measurements of the total protein content and paper electrophoretic analysis. Rheumatoid factors were studied by the Heterophile Absorbed Sheep Cell Test (SSC test), the FII Acryl Particle Fixation Test (FII AP test) and the FIIA Coated Tanned Sheep Cell Test (FIIA SC-test). A fluorescent antibody method was used in the search for antinuclear factors. Wassermann's, Meinicke's and Kline's reactions were used as serologic tests for syphilis. All laboratory examinations were carried out with the same methods and in the same laboratories as used in Leonhardt's study of SLE (10).

The authors personally interviewed all subjects as to earlier diseases and present state of health, with special regard to autoimmune diseases. The mother of the proband was subjected to additional physical and laboratory investigation.

Case report

A girl, born July 21, 1951, was first admitted in Jan. 1962 to the Department of Pediatrics, Central County Hospital, Kristianstad, for investigation of a high ESR (about 100 mm/1 hour, Strom). In 1960 the patient had had recurrent peritonsillar abscesses and tonsillectomy had been performed. Since summer 1961 the parents had noted that minor accidental wounds on the patient's hands were easily infected and healed slowly. In Nov. 1961 she had had a bout of impetigo, and in Dec. 1961 purulent conjunctivitis. In addition to this proneness to infections, the patient was said to have had small hemorrhages of her mouth for a couple of years. There was no history of abdominal pain or jaundice and no known exposure to infectious hepatitis. Perusal of the records of the outpatients' department revealed that already in 1957 the ESR had been abnormally high (30—53 mm/1 hour, Strom) without other signs of disease.

Physical examination revealed nothing remarkable except paronychia of the right thumb and slightly enlarged anterior cervical lymph nodes. The liver was not palpable. Laboratory studies showed an ESR of 55

TABLE I Sex and mean age of proband's relatives and of controls

Group	Sex	No	Mean age	S D	S E of mean
Paternal relatives	♂	15	40 5	19 93	5 15
	♀	8	33 5	21 26	7 52
Maternal relatives	♂	19	36 7	19 70	4 52
	♀	12	38 9	23 37	7 04
Controls I	♂	7	45 7	18 48	6 98
	♀	13	44 9	12 38	3 43
Controls II	♂	180	46 3	13 40	1 00
	♀	144	43 2	12 84	1 07

mm/1 hour (Ström) and analysis of the serum proteins by electrophoresis on paper showed a gammaglobulin value of 5 6 g/100 ml the gammaglobulin fraction representing about 50 % of the total serum protein Immunoelectrophoretic analysis (Assoc Prof C B Laurell Department of Clinical Chemistry Malmo General Hospital) showed that the gammaglobulin component consisted mainly of 7 S globulins Also the B_2M fraction was significantly increased but B A appeared to be within normal limits Two preparations for LE-cells were made and abundant typical LE-cells were seen SGOT was raised to 310 and 240 units on two determinations (normal range 10—40 units) and SGPT to 100 and 90 units (normal range 10—30 units) However the serum bilirubin level was normal 0 5 mg /100 ml and the direct van den Bergh reaction was negative Routine blood and urinalysis showed nothing abnormal The patient was sent home symptom free Diagnosis hyperimmune state of unknown cause

The patient was hospitalized again in Sept 1962 because of two months arthralgia and two weeks fatigue sore throat and low grade fever On examination she looked tired pale and slightly jaundiced The liver was palpated 3 cm below the right costal margin The spleen was not palpable Laboratory studies revealed an ESR of 63 mm/1 hour (Ström) massive hypergammaglobulinemia (6 3 g/100 ml) and a low serum albumin (2 0 g/100 ml) The serum bilirubin was 1 5 mg/100 ml and gave a positive direct van den Bergh reaction SGOT and SGPT were 335 and 83 units respectively LE-cells were found in 1 of 4 preparations made The patient was also found to have anemia with 7 3 g hemoglobin/100 ml and a red cell count of 2 9 mill /mm³ There was no reticulocytosis the serum haptoglobin was 50 mg/100 ml (normal range 30—190 mg/100 ml) and Coombs direct test was negative The fever lasted about 10 days and then disappeared spontaneously The patient left hospital at the parents request

In Oct 1962 she was re admitted after deterioration of her general condition She was markedly jaundiced and showed several spider nevi As before the liver was palpated 3 cm below the right costal margin The spleen was still not palpable Laboratory studies showed no essential changes since Sept 1962 except a rise in serum bilirubin to 4 mg/100 ml still with a positive direct van den Bergh reaction The LE-cell test was now negative The condition was regarded as typical for lupoid hepatitis and the patient was therefore treated with *prednisolone* 20 mg a day On this therapy she improved markedly She was no longer so tired her appearance improved and the spider nevi and liver enlargement disappeared The serum bilirubin fell to normal levels SGOT and SGPT

decreased to 98 and 55 units, respectively There was a decrease of the serum gammaglobulin to 3 8 g/100 ml and an increase of serum albumin to 3 3 g/100 ml

In Nov 1962 the girl was transferred to the Department of Internal Diseases, Malmo General Hospital, for supplementary investigation She still had signs of active liver disease with elevated serum transaminase levels (SGOT 122 and SGPT 51 units) The serum bilirubin was 1 1 mg/100 ml (positive direct van den Bergh's reaction), and the bromsulphalein test gave a retention of 18 % after 30 minutes (normal range 2—10 %) Blood clotting studies (Ass Prof I M Nilsson) revealed low levels of prothrombin (45 % — normal range 85—110 %), factor V (53 % — normal range 80—120 %) and fibrinogen (0 17 g/100 ml — normal range 0 26—0 34 g/100 ml), and studies of the fibrinolytic activity of the plasma gave evidence of a low content of inhibiting factors The serum haptoglobin was 15 mg/100 ml Test for LE cell phenomenon was negative The SSC test was borderline (16), but the FII-AP test was positive (80) The Wassermann, Meinicke and Kline tests were negative The thyroglobulin coated tanned red cell agglutination reaction was negative On testing the complement fixation reaction with thyroglobulin, the serum proved strongly anticomplementary There was moderate anemia (Hb 8 9 g/100 ml, red blood cell count 3 6 mill/mm^3) and a low serum iron level (29 μg/100 ml — normal range 55—155 μg/100 ml) and there was good response to oral iron therapy X ray of the esophagus produced no evidence of varicose veins Owing to the abnormal results of blood clotting studies liver biopsy was refrained from The patient was sent home on 20 mg prednisolone a day

From Dec 1962 to July 1964 the patient was followed up at the outpatients department of the Pediatric Clinic Kristianstad During this time she had been taking prednisolone 10—20 mg a day Her general condition had been excellent and she had not had jaundice, liver enlargement arthralgia or any bleeding tendency Laboratory studies showed a normal serum bilirubin during the entire period When the steroid doses were kept at 10 mg a day there was an increase of SGOT to 50—85 units and of SGPT to 35—58 units On a daily dose of 20 mg prednisolone, however, the serum transaminase levels were within the normal range The ESR had been normal or slightly increased, rarely exceeding 25 mm/1 hour (Ström) After the initial decrease of serum gamma globulin and increase of serum albumin no significant change in the concentration of these proteins had occurred

The *mother* of the proband, 43 year old, had always felt healthy and physical examination showed no abnormalities Routine blood and urinalysis were normal Liver function tests were likewise normal serum bilirubin 1 0 mg/100 ml (negative direct van den Bergh reaction), SGOT and SGPT normal bromsulphalein retention 5% after 30 minutes She had, however, hypergammaglobulinemia of 1 73 g/100 ml on repeated examinations the serum gammaglobulin was on the same level (1 7—2 0 g/100 ml)

On the whole, the other *relatives* of the proband were subjectively healthy II 20 (fig 1) a 41 year old female had chronic pyelonephritis which was clinically well controlled and her gammaglobulin was 0 97 g/100 ml at the time of examination I 3, a 68 year old male was known to have pernicious anemia Of the *deceased* members of the pedigree, II 17 had had chronic myeloid anemia Several relatives of the probands and some of their married partners complained of mild arthralgia but none had chronic deforming polyarthritis and none was known to have autoimmune disease of any other type

Results

Gammaglobulin

As the gammaglobulin levels tend to rise with age and to be somewhat higher in females than in males (16), the observed gammaglobulin values were adjusted for age and sex according to Leonhardt (10) The individual

TABLE II Means of the age-corrected gammaglobulin values of proband's relatives and of controls

Group	Sex	Total tested	Mean	S D	S E of mean
Paternal relatives	♂	15	0.92	0.12	0.03
	♀	8	1.01	0.16	0.06
Maternal relatives	♂	19	1.13	0.20	0.05
	♀	12	1.25	0.26	0.08
Controls I	♂	7	0.99	0.17	0.06
	♀	13	0.98	0.17	0.05
Controls II	♂	180	0.87	0.17	0.01
	♀	144	0.95	0.21	0.02

TABLE III Distribution among standardized intervals of the age-corrected gammaglobulin values in proband's relatives and in controls

Group	Total tested	Standardized gammaglobulin intervals			
		0	+1	+2	≥3
Paternal relatives	23	8	10	5	0
	%	34.8	43.5	21.7	0.0
Maternal relatives	31	4	7	9	11
	%	12.9	22.6	29.0	35.5
Controls I	20	11	3	5	1
	%	55.0	15.0	25.0	5.0
Controls II	324	173	99	42	10
	%	53.4	30.6	13.0	3.0

values were corrected to hold for the age class 40—49 years. The regression coefficient of 0.03 g/100 ml per 10 year interval calculated for 324 controls in the investigation of Leonhardt (10) was used for this purpose. The means of the age-corrected gammaglobulin values of the relatives and of the controls are given in table II. The age-corrected gamma globulin values were then divided into standardized intervals calculated from the mean and the standard deviation (SD) of each sex. For convenience only 4 classes were used for statistic comparisons.

Below mean	= 0
Mean to mean + 1 SD	= + 1
Mean + 1 SD to Mean + 2 SD	= + 2
Mean + 2 SD and above	≥ + 3

If distributed according to the normal curve 50.0 %, 34.1 %, 13.6 % and 2.3 % of a population of gammaglobulin values should fall within the standardized intervals in the order given.

The distribution among standardized intervals of the age corrected gamma-

TABLE IV. Distribution, among negative (−), borderline (+ −) and positive (+) results, of tests for rheumatoid factors in proband's relatives and in controls

Group	Total tested	SSC-test − (8)	SSC-test + − (16—32)	SSC-test + (≥64)	FII AP test − (0)	FII AP test + − (10—40)	FII AP test + (≥80)	FIIA SC-test − (0)	FIIA SC-test + − (8—64)	FIIA SC-test + (≥128)
Paternal relatives	23	20	3	0	20	3	0	20	3	0
	%	87.0	13.0	0.0	87.0	13.0	0.0	87.0	13.0	0.0
Maternal relatives	27	22	5	0	18	9	0	21	5	1
	%	81.5	18.5	0.0	66.7	33.3	0.0	77.8	18.5	3.7
Controls I	16	15	1	0	13	3	0	14	2	0
	%	93.7	6.3	0.0	81.3	18.7	0.0	87.5	12.5	0.0
Controls II	324	279	33	10	311	7	6	274	34	14
	[1] %	86.6	10.2	3.2	96.0	2.2	1.8	85.1	10.6	4.3

[1] SSC test and FIIA SC test was performed only in 322 controls.

globulin values of the groups examined is given in table III. Statistic analysis gave as follows (males and females were combined within each group, as they did not show any significant differences in gammaglobulin distribution).

	χ^2	DF	P
Between controls I and controls II	3.76	3	> 0.20
Between paternal relatives and controls II	4.51	3	> 0.20
Between maternal relatives and controls II	65.19	3	< 0.001
Between paternal and maternal relatives	13.11	3	< 0.01

Thus, *the distribution of gammaglobulin values of the married partners* examined (controls I, 20 individuals) did not differ from that of the controls used in the previous investigation of Leonhardt (controls II, 324 individuals). This further justifies the use of the latter control material in the present study.

The paternal relatives of the proband did not differ significantly from the controls regarding the distribution of gammaglobulin levels. *On the other hand, there was a highly significant* ($P < 0.001$) *difference between maternal relatives and controls, the former having a higher frequency of gammaglobulin values in the +2 and > +3 standardized intervals than the latter. Comparison between maternal and paternal relatives* consequently showed significantly ($P < 0.01$) higher gammaglobulin values among the maternal relatives.

Rheumatoid factors

The results of tests for rheumatoid factors are given in table IV. There was a tendency towards a higher incidence of borderline values in the maternal relatives of the probands than in the paternal

relatives and in the controls Owing to the smallness of the sample (groups of relatives and controls I), making age and sex adjustment difficult statistic evaluation was not undertaken

It is noteworthy that some *young* maternal relatives had borderline results in more than one of the tests for rheumatoid factors (fig 1) *III 19* 13 year-old female FII AP 40 FIIA SC 32 *III 20*, 20 year-old female SSC 16 FII AP 10, *III 21* 14 year old male FII AP 20 FIIA SC 64 *III 25* 14 year-old male SSC 32 FII AP 20 FIIA SC 32 *III 27* 23-year-old female SSC 16 FII AP 10 *III 28* 13 year-old female SSC 32 FII AP 10 Three of these individuals (III 19 III 20 and III 28) also had hypergammaglobulinemia Only one of the paternal relatives and one of the married partners (controls I) had borderline values in more than one of the tests for rheumatoid factors, these individuals were 75 and 55 years old respectively As known the incidence of borderline and positive tests for rheumatoid factors tends to rise with age (1) Thus the occurrence of such results in young individuals should be regarded with some significance

Tests for syphilis

All serologic tests for syphilis carried out in relatives and in controls were negative There was thus no false positive serologic reactor in this material

Antinuclear factors

The fluorescent test for antinuclear factors was strongly positive in the *proband* at the time of the study A positive test was also found in the *mother* of the proband One dubiously positive result was found in one of the controls all the other individuals reacted negatively

Discussion

The clinical picture exhibited by the proband fits in well with that described by Mackay et al (11) under the heading of lupoid hepatitis" Cases of lupoid hepatitis in *children* have been reported by several authors, e g Bearn et al (2) Page and Good (15), Willcox and Isselbacher (18), Grossman et al (4) and Mackay and Wood (12)

It is difficult to determine the exact onset of active liver disease in the proband, since jaundice increased gradually in the course of several weeks and was preceded by elevated serum transaminase levels for at least six months Before being jaundiced the patient had had arthralgia of two months' duration and spells of bleedings of the mouth for a couple of years During the patient's first stay in hospital abundant LE-cells were found in peripheral blood preparations but later on, when jaundice was marked no LE cells were found An elevated ESR had been known for at least five years and extreme hypergammaglobulinemia for about six months before the manifestation of jaundice This gradual piling up of symptoms and signs into the fully developed disease is strongly reminescent of SLE — this syndrome is known often to be preceded by various physical and laboratory abnormalities *inter al* by essential hypergammaglobulinemia (10)

As in SLE the treatment of choice in lupoid hepatitis is adrenocorticosteroid therapy (13 15) The progressive liver destruction seems to be suppressed by continuous administration of such drugs (5) — possibly by inhibition of autoanti

body production In our patient prednisolone obviously reversed the unfavourable clinical course, and it was possible to keep her symptom free on a maintenance dose A precise dose could be titered out which also kept the serum transaminase levels within the normal range (14)

Examination of the gammaglobulin levels in the proband's relatives disclosed that the maternal relatives had strikingly high levels Compared with the distribution of a large control population, the difference was highly ($P < 0.001$) statistically significant On the other hand, the paternal relatives did not differ from the controls regarding the distribution of gammaglobulin values The elevated gammaglobulin levels in the maternal relatives were probably not caused by environmental factors, since most of the relatives were not living together, and since they had a standard of living comparable to that of the paternal relatives who had the same gammaglobulin distribution as the controls It would thus appear that the proband had a pre disposition to overproduction of gammaglobulin inherited from her mother

Though the finding of familial hyper gammaglobulinemia in a single case does not warrant the conclusion that all cases of lupoid hepatitis have the same background, preliminary observations by Tiling et al (3) suggest that hypergammaglobulinemia might not be uncommon in relatives of patients with chronic hepatitis (their proband material including cases with different clinical pictures) The finding of a familial background of hypergammaglobulinemia in lupoid hepatitis would agree with what has been found in SLE (9, 10) and might be taken as evidence of pathogenetic mechanisms common to these two syndromes

Some importance might be attached to the finding of a positive test for anti nuclear factors in the mother of the proband and of borderline values for rheumatoid factors in several young maternal relatives of the proband The material was, however, too small for valid statistic evaluation of the incidence of these factors As for SLE relatives, Leonhardt's study (10) showed an increased incidence of antinuclear factors in such relatives, whereas the presence of rheumatoid factors in the relatives seemed to be dependent on the clinical picture of the corresponding probands (a significantly increased incidence of borderline and positive of tests for rheumatoid factors being found only in relatives of patients with suspected SLE)

It is notable that there were no cases of clinically manifest liver disease in the relatives of the proband Liver function tests were carried out only in the proband's mother, who had marked hyper gammaglobulinemia, but were found to be normal It is probable that the syndrome of lupoid hepatitis may develop only in the presence of a proper constellation of genetic predisposition to autoimmunity and eliciting environmental factors Thus e g viral destruction of hepatic cells might lead to excessive autoantibody formation and consequent self perpetuating liver disease in individuals with inherited hyperactivity of the gammaglobulin producing system It seems nevertheless likely that full blown lupoid hepatitis might

exist in two or more members of some families The occurrence of chronic hepatitis with manifestations also sugges tive of SLE in two sisters was described by Krook (6) Co-occurrence of SLE and chronic hepatitis was found in a few pedigrees reported by Leonhardt (10)

As a final remark the importance of recognizing familial hypergammaglob ulinemia in children as well as in adults should be stressed This condi tion may herald chronic autoimmune disease such as SLE or lupoid hepatitis

Summary

A case of lupoid hepatitis in an 11 year old girl is described The icteric phase had been antedated by hypergamma globulinemia for at least half a year and by elevated ESR for at least five years Investigation of the patient s pedigree disclosed strikingly high gammaglob ulin values in the maternal relatives The incidence of hypergammaglobuline mia in these relatives was significantly higher than in the paternal relatives and than in a control population It is proposed that lupoid hepatitis and system ic lupu erythematosus may be closely related and that both syndromes may have a genetic predisposition mani festing itself as familial hypergamma globulinemia

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Antazoline (Antistina®) as an Antiarrhythmic Agent

The Risk of Cardiac Arrest

By

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During recent years several reports have been published on the antiarrhythmic effect of antazoline (Antistina®) (1, 5 6, 7) Administered intravenously, the drug was found to be effective both against ventricular and against supraventricular tachycardia Serious complications have not been observed with the doses employed, which range from 2 to 5 mg of antazoline per kg of body weight The only side effects reported are dryness of the throat and drowsiness (5) When administered orally, antazoline seems to have only a slight antiarrhythmic effect

We have treated a fairly limited number of patients with antazoline However since we have encountered three cases of cardiac arrest in our small series we considered it imperative to present our experience in order to show that antazoline as most other efficient antiarrhythmic drugs may give rise to cardiac arrest and thereby may cause sudden death

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Material and methods

Seven patients were treated with antazoline intravenously They were patients with long standing attacks of supraventricular tachycardia Antazoline was administered in doses ranging from 2 to 8 mg per kg of body weight

In 3 of the 7 patients cardiac arrest occurred during or immediately after the administration of antazoline In one of the remaining 4 patients antazoline was administered intravenously during a total of about 50 attacks of supraventricular tachycardia Each time the tachycardia subsided immediately after the injection and no side effects were observed In 3 patients the tachycardia continued unchanged after injection of antazoline

Case reports

Case 1 A 20-year-old male with mitral incompetence and paroxysmal attacks of long standing supraventricular tachycardia, which previously had been extremely intractable However when administered intravenously antazoline had a prompt effect on the attacks of tachycardia Injections of 200—300 mg were given for approximately 100 attacks of

fibrillation or ventricular standstill This risk is marked when quinidine is administered intravenously (4), and it is not negligible even if the drug is administered orally (8) Similar severe reactions to antazoline have not been observed previously, and it was therefore believed that fairly liberal indications could be established for the use of this drug However, our experience shows that antazoline may also produce cardiac arrest

The arrests were so brief that perhaps the patients would have survived even if no resuscitation manoeuvres had been performed In one patient, cardiac arrest was due to ventricular standstill, whereas in the other two patients the mechanism is not fully clarified All three patients suffered from severe heart disease Two of them had just undergone major operations because of congenital heart disorders, and the third patient had considerable mitral incompetence with congestive heart failure The volumes of antazoline injected were 4 5 and 7 mg per kg of body weight respectively i e the doses given were smaller than the maximum dosage of 10 mg per kg of body weight recommended in the literature (2)

It must be concluded that in some patients intravenous administration of antazoline has a pronounced effect on supraventricular tachycardia but that this treatment may produce cardiac arrest and that the danger of this complication perhaps is particularly great in patients with serious myocardial disorders Consequently, intravenous administration of antazoline should only be used when weighty indications exist, and preferably only when facilities for rapid and efficient management of cardiac arrest are available

Summary

Three cases of cardiac arrest in connection with intravenous administration of antazoline are presented In all the cases heart action was re established after a very brief period of resuscitation, and none of the patients died It is concluded that antazoline should be given only when weighty indications exist, and preferably only when circumstances permit of resuscitation manoeuvres

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tachycardia over a period of one year, during which we have seen the patient

On one single occasion 200 mg of antazoline had no effect, and consequently another 200 mg was administered intravenously immediately afterwards At the end of the injection the patient lost consciousness and developed generalized convulsions There was no detectable pulse or audible heart action External cardiac massage was at once instituted, and after 10—20 sec the heart action was re-established The heart rate was about 150/min and the tachycardia did not subside till about 15 min had passed

At subsequent attacks, the patient was given a total of about 30 injections of 200—300 mg of antazoline Each injection resulted in prompt cessation of the tachycardia, and no side effects were observed

No evidence of brain damage was observed after the cardiac arrest

Case 2 A 10 year old female with Fallot s tetralogy On Sept 24th, 1964, she had a complete correction of the tetralogy during extracorporeal circulation

During the operation, complete atrioventricular block developed requiring implantation of electrodes in the left ventricle and application of an artificial pace maker for about one week

On Oct 6th, 1964, supraventricular tachycardia set in, and the patient was given digitalis On Oct 7th the tachycardia still persisted D C counter shock treatment was given with no effect When 200 mg of antazoline were injected intravenously nodal rhythm began, after one hour changing into sinus rhythm

On Oct 8th, there was another attack of supraventricular tachycardia, and antazoline was given once more but this time only 100 mg were administered intravenously During the injection the patient lost consciousness and convulsions occurred

During the attack, the cardioscope showed regular atrial action, but no ventricular complexes, and the patient had no detectable pulse The pace-maker was switched on, and ventricular action started immediately Later nodal rhythm and complete atrioventricular block developed, but in the course of some days sinus rhythm started

The patient did not present any signs of brain damage after the cardiac arrest

Case 3 A 30-year-old male with atrial septal defect In 1956, the patient underwent surgery by Bjork Sondergaard's method The defect was not repaired during this operation, and on May 26th, 1964, a further operation was done during extracorporeal circulation Prior to the latter operation the patient had sinus rhythm Three days later, on May 29th he developed atrial fluctuation and consequently, treatment with digitalis was instituted On May 31st, the ECG revealed supraventricular tachycardia with a ventricular rate of 150

It was decided to give antazoline intravenously During the injection the heart rate decreased slowly to 100 A total of 250 mg of antazoline was given Immediately afterwards the patient lost consciousness The eyes deviated upwards, the heart action was inaudible and the pulse absent

External cardiac massage was at once instituted In the course of 10—20 seconds heart action was re-established and the heart rate was again 150 By combined treatment with quinidine and electric counter shock (D C) sinus rhythm was established

There was no evidence of permanent brain damage after the cardiac arrest

Discussion

Animal experiments have shown that quinidine and antazoline both increase the refractory period of the myocardium (3), and clinically both agents are used as antiarrhythmics The therapeutic value of quinidine is somewhat limited, however, by the serious side effects of the drug First and foremost there is a risk of cardiac arrest due to ventricular

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Comparative Trends, in Four Scandinavian Countries, of Mortality from Atherosclerotic and Degenerative Heart Disease During the Years 1952—1960

By

G Biorck and G Bylin

In recent years both the scientific and the lay press have placed considerable emphasis upon the apparently rapid increase in mortality from cardiovascular diseases in general and from atherosclerotic heart disease in particular The prevailing tendency has occasionally been depicted as an "epidemic" (6) This impression has been derived from over all figures including all age groups but it has often been specifically stated that the increase has been very marked in the younger age groups Such a development has not been the experience in Swedish statistics however We have therefore attempted to analyse in greater detail the trends in mortality from certain cardiovascular diseases in four Scandinavian countries during a period of 9 years extending from 1952 to 1960 inclusive Further impetus to such a comparative study has been the previously noted marked difference in mortality from atherosclerotic heart disease between Finland and Sweden as analysed by Blomqvist and Biorck (2)

We have therefore examined the trends in mortality within the statistical group A 81 acc to the World Health Organization classification (which is synonymous with group B 26) and which includes atherosclerotic and degenerative heart diseases (table I) Figures from Denmark, Finland Norway and Sweden for each year of the period 1952—1960, divided with regard to sex and 5 year age groups were derived from the annual volumes published by the WHO (8) for the respective years We have chosen to start with the year 1952 although 1951 was actually the first year in which the new international classification was used

We have also attempted to study possible differences between the cities and the country side in Sweden and for this particular purpose we have had access to figures from Stockholm, Gothenburg and Malmo the three largest cities in

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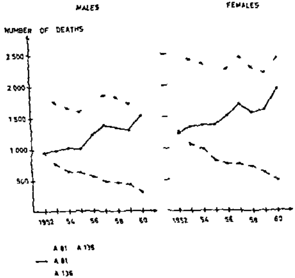


Fig 1 Number of deaths in A 81 A 136 and A 81 + A 136 > 85 years

We have also investigated the number 795 2 of the international list which de notes sudden death for unknown reasons, but it has become quite clear that the number of cases in this group in the Swedish statistics is so small that it can hardly influence the major trends which we are studying In Norway however this group has doubled from 353 in 1952 to 634 in 1960 This development has in its turn, occurred simultaneously with a decrease in A 136 Of greater interest is the group A 136 which contains number 794 – senility without mention of psychosis

Analysis of the co variance of A 81 and A 136 in Sweden has shown as evidenced by table II and fig 1 that it is only in age groups above 80 years that the figures for A 136 are of sufficient magnitude to interfere with the trends in A 81 It is evident that in the age groups above 85 years the increase in A 81 is caused in part by a tendency to discard the diagnosis A 136 In every comparison between the Scandinavian countries with regard to trends and particularly when the trends do not agree special attention must be directed towards this factor as a possible source of such disagreement However, information from Norway (9) has made it doubtful to what extent this explanation is valid for the difference in the trends which — as will be shown later — manifests itself between Norwegian figures and those from the other Scandinavian countries

For everyone working with interna tional studies on the causes of death the lack of precision in the basic figures as well as local differences in handling the statistical material are matters of considerable concern On the other hand, we have no other possibility than to accept the figures provided and to read them with a critical mind Another difficulty encountered in our work is the fact that the number of deaths in ages

TABLE I

A 81 ~ B 26 =	420 =	*Morbus arteriosclerotieus cordis*
	421 =	*Endocarditis chronica non rheumatica*
	422 =	*Alia degeneratio cordis*
A 136 =	794 =	Senilitas psychosi non indicata

Sweden, and the county of Jamtland, situated in the middle of Sweden near the Norwegian border Relevant figures here have been obtained through the courtesy of the Central Bureau of Statistics in Sweden It is our hope that this comparison will be useful in a further comparison of a similar type between cities and country side, centered on Norway and Finland

The group number A 81, which is identical with B 26 in the WHO international list, includes the diagnostic numbers 420 = atherosclerotic heart disease (coronary heart disease), 421 = chronic non rheumatic endocarditis and 422 = other myocardial degeneration The latter is a rather poorly defined group 421 is a very rare diagnosis which does not lead to great difficulties in the statistical analysis On the other hand, 422 has over the last few years been generally considered a "waste paper basket" with a very doubtful content Generally, one can assume that cardiac failure which can not be ascribed to congenital, valvular or hypertensive heart disease, and which has not given rise to typical symptoms such as angina pectoris or myocardial infarction, in many instances has been coded as 422 Recent studies (1), however, have indicated that this group may be much more homogeneous than formerly believed There is no doubt that a further close examination of the 422 group may reveal facts which have hitherto been concealed or muddled

TABLE II Number of deaths in A 81 and A 136 for the years 1952 1956 and 1960

	Year	Code Number	<69	70–74	75–79	80–84	>85
Males	1952	A 81	3,092	1 343	1 531	1 121	953
		A 136	11	56	184	424	954
	1956	A 81	3 677	1 710	1 843	1 575	1 218
		A 136	7	36	93	242	557
	1960	A 81	4 284	2 004	2 158	1 965	1 519
		A 136	4	18	56	94	306
Females	1952	A 81	1 604	1 235	1 500	1 308	1 240
		A 136	20	69	255	556	1 271
	1956	A 81	1 650	1 392	1 825	1 673	1 498
		A 136	13	34	128	307	741
	1960	A 81	1 706	1 527	1 991	1 985	1 916
		A 136	8	21	65	171	473

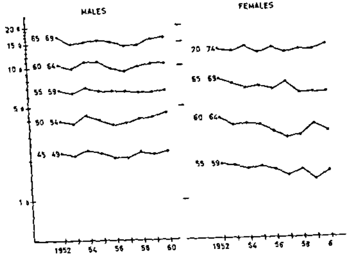


Fig 4 Finland Death rates in B 26 per 1 000 inhabitants

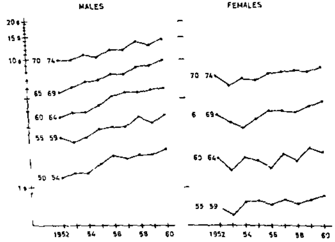


Fig 5 Norway Death rates in B 26 per 1 000 inhabitants

in the range of 50—74 years as being of greatest clinical interest Fig 2 presents a comparison between the four Scandinavian countries in age groups 60—64 for men and women

The figure shows that with regard to both men and women Finland is markedly above Denmark Norway and Sweden Denmark and Sweden show approximately the same levels, whereas the levels in Norway at the beginning of the period are clearly below those of the other countries, but later on the Norwegian curve reaches or even crosses the levels for Denmark and Sweden Although only the group 60—64 is dis

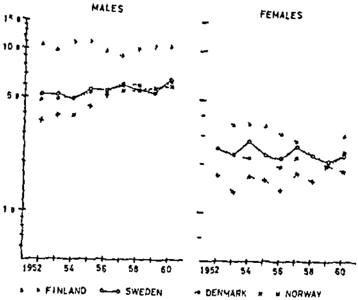


Fig 2 Death rates in B 26 per 1,000 inhabitants 60—64 years

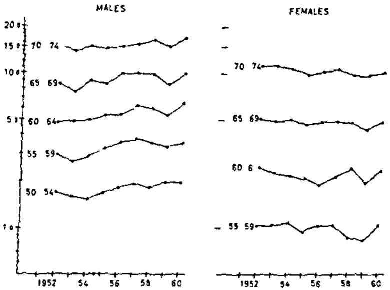


Fig 3 Denmark Death rates in B 26 per 1,000 inhabitants

below 50 years is so small that they hardly make reliable material for statements about trends

It is also evident from the figures that variations between consecutive years are fairly considerable The choice of the first and the last year may therefore influence the trend, in particular, the figure for the last year may give rise to spurious impressions

The graphs that will be presented in the following express mortality as annual number of deaths per 1,000 of the population, plotted semilogarithmically against the years in question We have considered for presentation age groups with

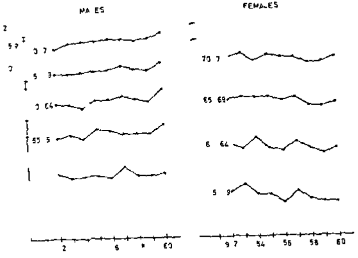


Fig 6 Sweden Death rates in B 26 per 1 000 inhabitants

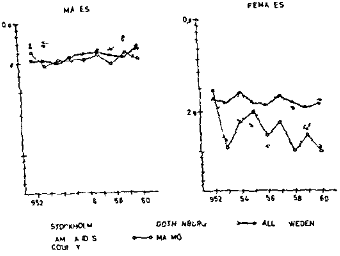


Fig 7 Death rates in B26 per 1 000 inhabitants 60–64 years

cause the absolute figures there are relatively small Nevertheless there appear to be distinct differences between the three cities on the one hand and Jamtland on the other

Within the group of cities Stockholm shows the highest mortality for men closely followed by Gothenburg Both these cities are above or at the level of the mortality figures from all Sweden, while Malmo is somewhat below that level On the whole the level for the years 1952–60 is constant or slightly falling for the three large cities despite the fact that the development in the whole country is in the opposite direction,

played in fig 2, the curves for age-groups 55—59 and 65—69 do show fairly similar trends, but of course at different levels Below age 55, Denmark has a steeper upward slope than Sweden and approaches that of Norway

As for the individual countries, it can be shown that in Denmark the mortality in B 26, during the period studied, seems to have been constant for women in the age-groups 55—69 years — which may be of the greatest interest In males there is a slight increase of the trend, particularly in the age-groups 40—64 years Low mortality rates are observed for the years 1953 and 1959 for Danish men, similar dips are also observed for Swedish men (1954 and 1959) High values are found in Denmark and Sweden for the year 1957, which was a year with much influenza

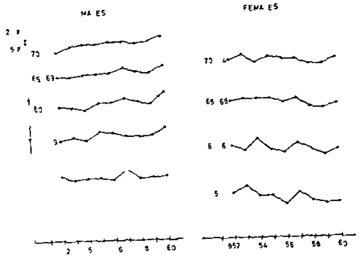
In Finland, mortality for B 26 in males of all age groups seems to be approximately twice as high as that in Denmark and Sweden For men, the values are practically constant during the period of observation, whereas for the women the trend is towards a slight fall, if anything, up to the age-groups above 70 years In higher age-groups, in contrast, the mortality is increased, this is particularly true for the group over 85 years of age

In Norway on the contrary, the figure shows a steep upward slope in most age groups studied This increase is most manifest in the age-groups 50—70 years for the men, and here the trend is markedly stronger than in the women At the beginning of the period, in 1952, the Norwegian figures in all age groups were below those in Sweden By 1955 the Norwegian curve crossed the Swedish figures for men in age groups below 54, and since 1957 also the values for age groups 55—59 years have been higher than those in Sweden The age-group 60—64 years at the end of the period was at the Swedish level, while the Swedish figures for higher age groups at that time still surpassed the Norwegian ones With regard to women, the Norwegian figures are still below the levels of the other three Scandinavian countries This situation, however, can soon change, because the Norwegian trend is rising, whereas the trend in the other countries and in Sweden in particular is falling

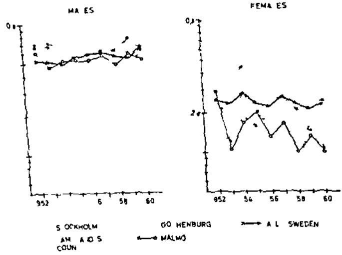
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The total mortality from all causes for men has fallen in age groups 40—54 years and above 54 years it has been constant For women it has fallen in all age-groups This means that although we appear to have a favourable tendency for the mortality in B 26, the relative percentage of deaths from B 26 as related to deaths from all causes is increasing

We have also had reason to look into the trends experienced in Stockholm, Gothenburg and Malmo as well as in the county of Jamtland The situation in Jamtland presents some difficulties be-



F g 6 Sweden Dea h ra es n B 26 per 1 000 inhab tan s



F g 7 Death ra es n B26 per 1 000 inhab tants 60—64 years

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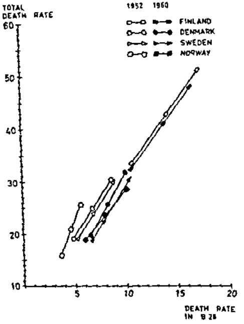


Fig 8 Coronary and total death rates per 1 000 population Men 60—64 and 65—69 years of age 1952 and 1960

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7 In view of the imminent revision of the international classification of causes of death it is very *important to analyse* the mutual relationship of the two components within the group B 26 viz 420 atherosclerotic heart disease and 422 other myocardial degeneration For example, previously reported studies of the calcium content of drinking water and the mortality in cardiovascular diseases (1) has revealed a highly significant negative correlation between calcium content of water and the mortality from the group 422 Separate studies of 420 and 422 are obviously *indicated* In Holland (4) as well as in England (5), there has been a gradual change where

particularly in the highest age-groups In Jämtland, the mortality in B 26 for the year 1952 was far below the mean for the country as a whole, but in 1960 Jämtland had surpassed the average for ages below 64 years For age groups 50 years and above, the trend in Jämtland is markedly rising and this rise is greater than for the country as a whole

With regard to women, there is the same difference in trends between the three cities and Jämtland although the levels as such are not greatly different Gothenburg is here approximately on the same level as Stockholm The trend is falling in all age-groups except the oldest one where it is horizontal It coincides with the average trend for the whole country up to age 69 After that age it is falling in the cities and horizontal in all Sweden Over 65 years it is horizontal in the cities but rising in the country as a whole Jämtland is showing a falling trend up to 69 years but thereafter there is a marked rise

It thus appears as if there were two different tendencies within the country in the cities an essentially unchanged or — with regard to the women — decreasing mortality rate whereas in Jämtland there is a marked increase beginning at upper middle age and continuing thereafter In as much as the trend for the country as a whole with regard to men is characterized by a certain increase in the higher age groups, it is tempting to believe that the figures from the county of Jämtland may represent a development within some Swedish country districts which statistically counteracts the opposite trend in the cities It may also well be that the change in the use of nomenclature from A 136 to A 81 has manifested itself later in the countryside than in the cities

An attempt to summarize the findings suggests the following observations

1 The impression of a rapid increase in mortality from atherosclerotic heart disease probably arises largely from the use of compound figures, in which the oldest age-groups play a dominant role When the figures are broken up with regard to sex and 5-year age groups, a much more varied picture presents itself

2 The high level of mortality in B 26 in Finland, as well as in mortality from all causes, is again demonstrated The reasons for this are unknown but certainly further study is warranted

3 In Denmark, Finland and Sweden there has been no alarming increase in the mortality in B 26 during the nineteen-fifties, at any rate in age-groups below age 70 The trends for men are either constant or slightly increasing, for women they are constant or falling There is no evidence of increased mortality in very young age groups in Finland or Sweden, while Denmark shows some increase for men 40—64 years old

4 Within Sweden the trend seems to have come to a standstill in the three largest cities, whereas a country area as instanced by the county of Jämtland shows a markedly increased trend for men particularly after the age of 50 and for women from 70 years onwards It is also possible that other rural areas may display a similar tendency and that it is such a development that maintains the increasing trend for the country as a whole in higher age groups despite tend-

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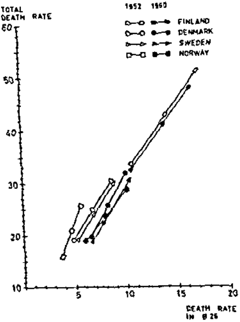


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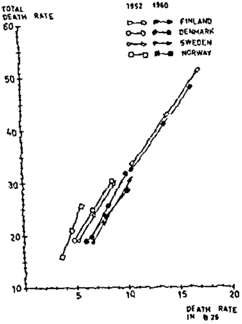


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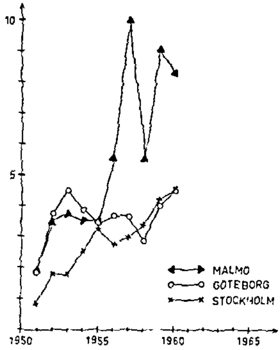


Fig 9 Ratio 420/422

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However, this shift in diagnosis does not influence the total group B 26 or A 81, although it certainly warrants consideration in other connections

Any interpretation in biological terms of the results presented above should be approached with much caution Reference may, however, be made to a recent study by Campbell (3), who has studied *similar problems in Great Brit*ain where there has been an increase in the mortality from heart disease, and from ischaemic heart disease in particular This has been rather rapid since 1927 and, according to Campbell it can be presumed to continue to 1973 from which point onwards there will be no great change at least until 1990 This *prophecy is based* upon the concept of "postponed mortality" in generations who otherwise should have succumbed to infectious diseases or diseases of childhood If this interpretation is correct, it is possible that we now are witnessing a phasic displacement in the mortality trends, which in a way *may correspond* to what Blomqvist and Biörck (2) earlier *observed for the relative levels in death* rates between e g Finland and Sweden *In only* one area *of the mortality* statistics in England has there been, during the last ten years, a steady state and this is with regard to coronary heart disease in physicians It is also worth mention in this connection that the trend in the mortality from all cardiovascular and kidney-diseases *together in the* United States (7) at the end of the nineteen *fifties — contrary to what is often stated* — has shown a decrease, not only for the coloured and *for the female* population, where this trend had been observed already earlier but also for white men where one has reason to believe that B 26 plays a very great role

When our observations are compared with these findings from other parts of the world it is reasonable to make a pause and reconsider the situation Clinical studies from our department have earlier pointed to the fact that the *mean age in patients with myocardial in*farction admitted to hospitals in our country *has been rising over the years* while we have had no real evidence that the frequency among *young people was* on the increase to any serious degree it least The present figures seem to substantiate such an impression Diseases of

the myocardium, either atherosclerotic in origin or from other causes, certainly constitute a very large part of the morbidity picture in any civilized population It may, however, be that clinicians should aim at a better separation of different types of disease, perhaps of different aetiologies, in different age groups and in both sexes In addition, the present study indicates the importance of analyses directed particularly towards elucidation of the causes of the differences between e g Finland and Sweden on the one hand, and Norway and Sweden on the other hand

Summary

On the basis of available official mortality statistics, a study of the trends in mortality from atherosclerotic heart disease (B 26) in the four Scandinavian countries over the years 1952—1960 has been performed This study has shown that there are differences in the pattern Whereas Denmark and Sweden show very little variation over this period Norway has a different trend The Norwegian figures start below the Danish and Swedish but reach or surpass them in at least some age groups during the period of observation Finland, on the other hand maintains a much higher level but this level is fairly constant during the period of observation Within Sweden there are no marked changes observed with the exception of the very high age groups, not even in the three largest cities, whereas a rural county shows a considerable increase also in somewhat lower age-groups Possible interpretations are discussed and areas for further investigations indicated

Acknowledgement

This investigation was supported by Folksam and Yngve Johnson s fund

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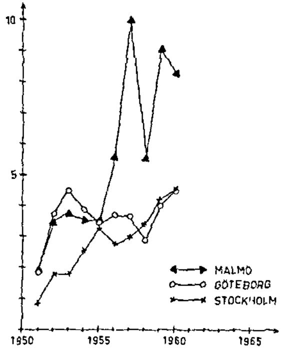


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From the Medical Department of the Kivela Hospital Helsinki, Finland

Furosemide (Lasix®) as a Diuretic

By

PENTTI PELTOLA[1]

In an earlier investigation (5) the author noted the common appearance of secondary hyperaldosteronism in congestive cardiac failure during long term use of saluretics The water and sodium excretion diminished, edemas began to increase and the patients developed hypokalemia even without a distinct increase in the excretion of potassium The symptoms soon improved through the administration of either aldosterone antagonists daily or saline on the days of treatment with diuretics the aim being to correct the excessive loss of sodium which seemed to be one of the major causes of the hyperaldosteronism during this treatment

As a too intensive natriuretic effect as compared with water diuresis thus does not seem beneficial in the treatment of congestive heart failure we tried in connection with a clinical study of a new diuretic to compare various saluretics for their efficacy in this respect The reference substances used in the study were chlorthiazide hydrochlorthiazide chlorthalidone and the new preparation furosemide (Lasix®, Hoechst) or 4 chlor-N-(2 furylmethyl) 5-sulfamoyl anthranilic acid

Submitted for publication January 11 1965

Material and methods

The series consisted of 90 furosemide treated patients with congestive heart failure originating from cardiosclerosis or valvular disease In 22 of these the other oral diuretics had lost their effect and signs of secondary hyperaldosteronism were apparant In addition 20 cases of liver cirrhosis with ascites were treated with furosemide The therapeutic dose was 25—120 mg/24 hours and the longest treatment time 1 1/2 years In addition 20—40 mg of furosemide was given intravenously to 25 patients with acute pulmonary edema The other comparative series consisted of 20 patients with congestive cardiac failure treated with chlorthalidone (dose 0 1—0 2 g/24 hours) 20 patients treated with chlorthiazide (dose 0 5—2 0 g/24 hours) and 20 patients treated with hydrochlorthiazide (dose 25—50 mg/24 hours)

The patients were kept on a diet in hospital the sodium intake being 100—120 mEq/24 hours and potassium intake 40—

[1] Present address Kivelä Hospital Helsinki

TABLE I Sodium excretion per liter of urine with different diuretics during the first 7 days of treatment in congestive cardiac failure The difference between furosemide and the other diuretics is statistically significant ($p < 0.01$)

	No	mEq/l
Hydrochlorthiazide	20	109 5
Chlorthalidone	20	104 8
Chlorthiazide	20	101 3
Furosemide	40	91 4

TABLE II Potassium excretion per liter of urine with different diuretics during the first 7 days of treatment in congestive cardiac failure The difference between the diuretics was not statistically significant ($p > 0.05$)

	No	mEq/l
Hydrochlorthiazide	20	56 3
Chlorthalidone	20	45 9
Chlorthiazide	20	53 7
Furosemide	40	41 9

Furosemide given intravenously in acute pulmonary edema

In 23 of the 25 cases of acute pulmonary edema in congestive cardiac failure a rapid improvement was seen 10—20 minutes after the intravenous injection of furosemide without any other treatment No harmful side effects were observed

The effect of the various diuretics on the excretion of electrolytes and water

The 24 hour excretions of water and electrolytes varied greatly with the different diuretics and doses used In general, however, the water diuresis was most pronounced with furosemide When the sodium concentration per liter of urine was calculated, a difference was observed between the preparations as can be seen from table I

Graphically a difference can be demonstrated between say, chlortahlidone and furosemide in the sodium concentration of urine of patients with a very similar water diuresis (fig 1) The marked natriuresis with chlorthalidone during the first week of treatment diminished quickly during continued therapy, and the aldosterone determination on the 10th day showed an aldosterone excretion about 50 per cent above normal The furosemide induced natriuresis was largely unchanged throughout the treatment and no signs of hyperaldosteronism were seen, aldosterone excretion was not increased

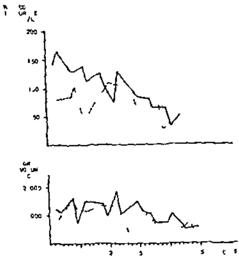


Fig 1 Urine volume and sodium content per liter of urine in patients treated with chlorthalidone and furosemide
Furosemide
Chlorthalidone –

60 mEq/24 hours. The digitalis dosage and rest of the treatment were kept unchanged during this study. The sodium and potassium concentrations in plasma and urine were determined daily, by flame photometer, as were body weight and urine volume. The urinary sodium and potassium excretions were calculated per liter of urine.

As liver function tests, thymol turbidity and bromsulphthalein retention tests were performed repeatedly in 15 cases of congestive cardiac failure even after treatment over one year with furosemide.

Results

Long-term furosemide therapy

A good diuretic effect with disappearance of the edemas was achieved in 87/90 of the patients with congestive cardiac failure and 19/20 of the patients with liver cirrhosis. In only 5 cases was it necessary to use the maximum dosage to ensure the effect. In one case of congestive cardiac failure without any distinct increase in diuresis, the patient had a chronic adhesive pericarditis with extensive edema of the liver and the lower limbs. In two other cases the diuretic effect disappeared after one to two weeks of treatment. Furosemide was also effective in 20 of the 22 cases in which the other saluretics had lost their effect, only in two cases were aldosterone antagonists necessary to establish the diuretic effect.

The one patient with liver cirrhosis in whom no diuretic effect was observed had pronounced hypoproteinemia (3.9 %). Yet a diuretic effect was established e. g. in 5 other patients with cirrhosis whose plasma protein was low 4.5—5.3 %.

Hypokalemia developed during furosemide therapy in two cases of congestive cardiac failure, the lowest plasma potassium value recorded was 3.5 mEq/l, but the condition cleared with continued therapy without addition of potassium to the diet. The same was seen in the hypokalemic patients previously treated with other saluretics. Two of the patients with cirrhosis of the liver developed hypokalemia ad 3.2 mEq/l, but the condition was not aggravated during continued therapy and the potassium values rose to 3.5 and 3.8 mEq/l. The hypokalemia established in three patients with cirrhosis of the liver prior to furosemide therapy was not aggravated during therapy, on the contrary, it disappeared in one case without addition of potassium.

No change was observed during furosemide therapy in the glucose balance of 10 diabetics in the congestive cardiac failure group, an indication of this was that the need of oral antidiabetic treatment and of insulin did not change during furosemide therapy. There was not a single episode of gout during therapy. The mean concentration of uric acid in the plasma before and during treatment in 10 cases rose from 5.5 to 6.9 mg %, but the difference was not statistically significant.

The long-term (1–2 years) use of furosemide in 15 cases of chronic congestive cardiac failure produced no side effects except for the nausea experienced by 2 patients, which was so mild that the therapy could be continued. Occasional thrombocyte determinations in 20 cases gave normal findings. The liver function tests performed gave normal results.

obviates the necessity for the administration of potassium The stimulating effect of potassium on the aldosterone excretion (1) can thus be avoided

A possible explanation for the difference between furosemide and other diuretics in electrolyte and water diuresis might be the different site of action Stop-flow studies (6) and micropuncture studies of the renal tubules (2) show that the effect of furosemide is on both the proximal and the distal tubules and on the loop of Henle, while the influence of the other oral diuretics is mainly on the proximal tubules

The rapid diuretic effect of furosemide that begins less than 10 minutes after an intravenous injection (8) seems to make it very suitable for the treatment of acute pulmonary edema

Diabetes seems not to be affected by furosemide The possible increase in the uric acid level in blood needs further observation

Summary

Long term treatment with furosemide (4 chlor N (2 furylmethyl) 5 sulfamoyl anthranilic acid Lasix® Hoechst), a new oral diuretic was studied With the use of furosemide for periods of more than 1 1/2 years and even by 90 patients with congestive cardiac failure and 20 patients with cirrhosis of the liver there was loss of diuretic effect decreased natriuresis and hypokalemia in only 3/90 cases of the former and 1/20 cases of the latter group The same symptoms taken as signs of secondary hyperaldosteronism were more common with other diuretics and usually disappeared when furosemide was used instead of them

Comparison between furosemide and certain other oral diuretics showed that furosemide sharply increased the sodium but especially the water diuresis per 24 hours, so that the sodium content per liter of urine was smaller than with hydrochlorthiazide, chlorthalidone and chlorthiazide The potassium excretion per liter of urine was similar with the various diuretics and corresponded to the potassium intake Hypokalemia was nevertheless more common in cases treated with the other diuretics, and was interpreted as a sign of anomalous distribution of potassium in the body caused by increased aldosterone effect and not as direct evidence of potassium deficiency

In accordance with the earlier studies by the present author the smaller natriuresis per liter of urine with furosemide might explain the less common appearance of signs of secondary hyperaldosteronism with this drug

Furosemide seems not to affect the glucose tolerance The possible effect on uric acid level in blood needs further evaluation The rapid onset of the effect of intravenous furosemide makes it effective in the treatment of acute pulmonary edema

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The potassium excretion per liter of urine with the different diuretics is tabulated in table II

The effect of various diuretics on the plasma sodium and potassium values

Occasional plasma sodium values below 137 mEq/l were seen before the appearance of hypokalemia Hypokalemia occurred in spite of the fairly uniform potassium excretion in 3/20 of the hydrochlorthiazide, 5/20 of the chlorthalidone, 2/20 of the chlorthiazide and 2/90 of the furosemide group of congestive cardiac failure These changes were generally associated with diminished sodium excretion and disappearance of the diuretic effect, all taken as signs of secondary hyperaldosteronism

Discussion

Furosemide proved to be an effective diuretic in clinical use Its daily long-term use rarely resulted in the electrolyte disturbances which are rather common with the other oral diuretics Its administration appeared to be fairly safe also in cirrhosis of the liver When other diuretics had lost their effect and symptoms of secondary hyperaldosteronism with consecutive electrolyte disturbances appeared, a change to furosemide usually restored diuresis and resulted in the disappearance of the disturbances mentioned without an eletrolyte supplement in the diet

Furosemide seemed to cause a liberal increase in the 24 hour sodium excretion but an even greater increase in water diuresis, with the sodium concentration per liter of urine remaining smaller than with thiazides and chlorthalidone This observation agrees with the findings of some other authors (3) The relatively smaller sodium excretion per liter of urine with furosemide might well in fact be an explanation of the less frequent appearance of secondary hyperaldosteronism due to an excessive loss of sodium (5) The rather short duration, a few hours only (3), of the effect of furosemide may also be a factor in preventing an excessive 24 hour excretion of sodium and thus working against the appearance of secondary hyperaldosteronism

The potassium excretion seemed to remain fairly constant with the various oral diuretics studied In spite of this, hypokalemia appeared more often with thiazides and chlorthalidone It can thus hardly be regarded as an indication of a real potassium deficit of the organism, but rather of aldosterone effect mentioned above It has also been shown that aldosterone administered to patients with congestive heart failure results in a decrease of sodium and urine excretion without an increase in potassium excretion (4), but yet may lead to hypokalemia (Peltola, in press) Studies with radioactive potassium have shown, in fact, that hypokalemia in congestive cardiac failure may develop without a demonstrable change in the exchangeable potassium in the body (7) In normal persons, in contrast, lowered natriuresis is accompanied by increased potassium excretion during aldosterone effect, readily resulting in a reduction in the amount of exchangeable potassium The rare occurrence of hypokalemia during the treatment with furosemide generally

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Chromosome Aberrations in Human Cells Induced by Thalidomide in Vitro

Preliminary Report

By

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During the past few years it has been shown that X radiation (2, 8) and treatment with several cytotoxics (7, 9) in vivo and in vitro are capable of producing chromosome aberrations in human cells As X radiation as well as some of the cytotoxics are known to be teratogenic (3 10) in man the following experiments were performed to ascertain whether thalidomide is able to produce chromosome aberrations in vitro

Materials and methods

Human lymphocytes from three healthy donors were cultured according to a slight modification of the method of Moorhead et al (6) As thalidomide is barely soluble in water 25 mg of the drug was first dissolved in 5 ml dioxane and 995 ml distilled water was added The solution was sterilized by Seitz filtration Two groups of cultures from each person were set up simultaneously and terminated after 72 hours

1 Cultures treated with thalidomide 1 μg/ml (200 μl of the solution added)
2 Control cultures with 200 μl of a solution containing the same amounts of dioxane and distilled water as above but without thalidomide added

Submitted for publication March 30 1965

Results

The results of the experiments are given in table I Most of the aberrations observed were chromatid and isochromatid gaps and breaks with formation of small acentric fragments A few deletions and translocations were seen

Discussion

Recently a few reports of chromosome investigations of children with anomalies due to thalidomide have appeared Hughes et al (5) were not able to

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TABLE I Effect of thalidomide on chromosomes of cultured human lymphocytes

Donor	No of cells analyzed	No of cells with chromosome injuries	Nature of chromosome injuries				
			Chromatid and isochromatid gaps	Breaks	Acentric fragments	Deletions	Translocations
P H	50	13	4	1	6	1	2
Control	50	1	1	—	—	—	—
G H	50	12	6	6	3	2	—
Control	50	4	—	1	1	2	—
K J	50	17	18	2	4	—	1
Control	50	5	2	1	2	—	—

demonstrate any chromosome aberrations in two patients examined In a male child with multiple anomalies, Benda and Baughman (1) demonstrated slight aneuploidy and multiple structural abnormalities of the chromosomes

After a single dose of 150 mg thalidomide, Green and Benson (4) found concentrations of up to 1 5 μg/ml plasma in man The thalidomide concentration, 1 μg/ml, used in the present study thus appears to be very close to the plasma concentrations obtained after pharmacological use of the drug

The applicability of chromosome studies of proliferating human lymphocytes in vitro after drug addition as a rapid and convenient screening technique for chemical damage to the genetic material deserves further exploration

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